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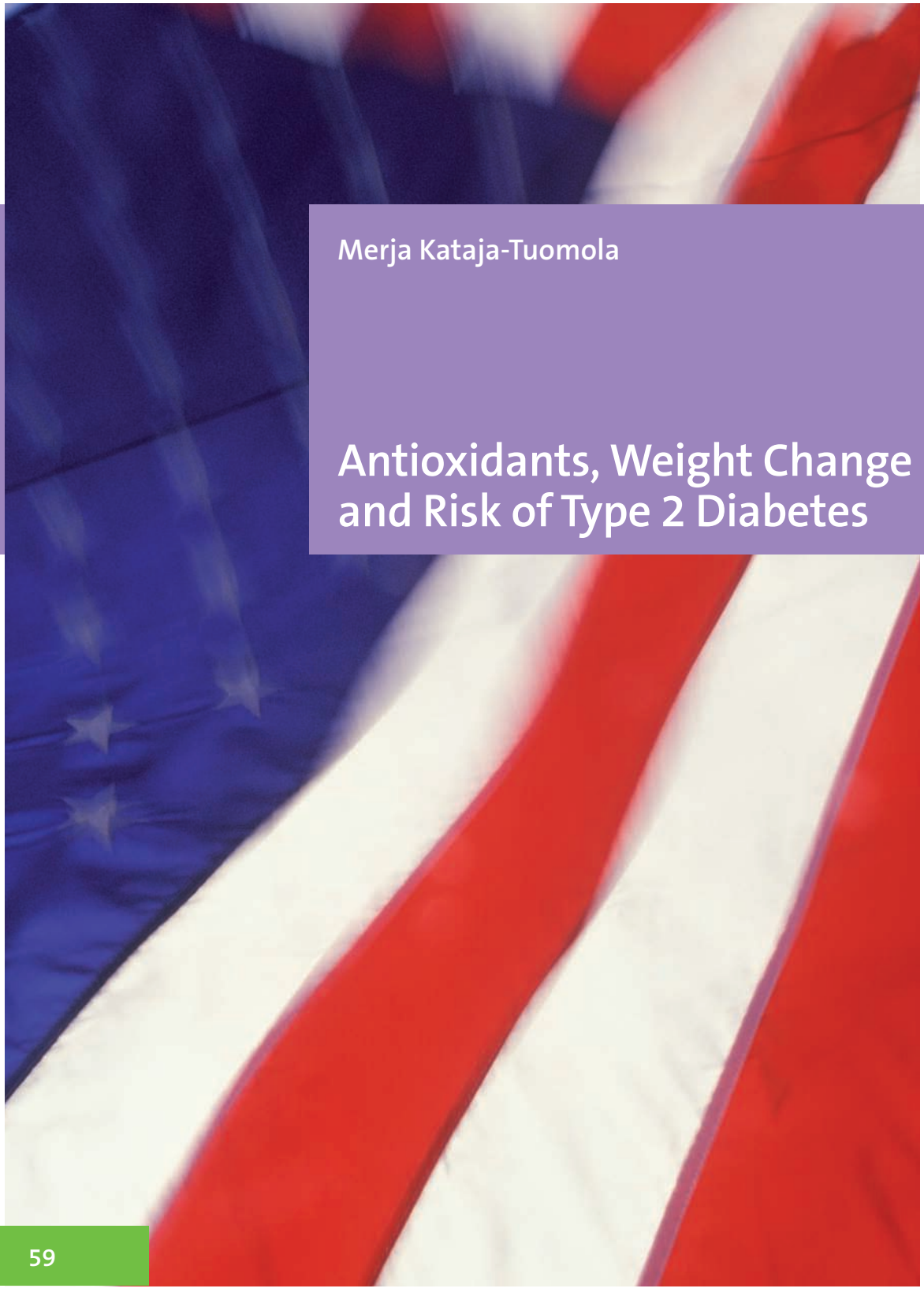

RESEARCH

Merja Kataja-Tuomola

Antioxidants, Weight Change and Risk of Type 2 Diabetes

Research 59

Merja Kataja-Tuomola

Antioxidants, weight change and risk of type 2 diabetes

Academic Dissertation

To be presented with the permission of the Medical Faculty
of the University of Helsinki,
for public examination in the Arppeanum, Helsinki University Museum,
on June 11, 2011, at 12 o'clock noon.

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To my twin sister Helena

Abstract

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The incidence of type 2 diabetes has increased rapidly worldwide. Obesity is one of the most important modifiable risk factors of type 2 diabetes: weight gain increases and weight loss decreases the risk. However, the effects of weight fluctuation are unclear since the findings of published studies are inconsistent.

Reactive oxygen species are presumably part of the complicated mechanism for the development of insulin resistance and beta-cell destruction in the pancreas. The association of antioxidants with the risk of incident type 2 diabetes has been studied in several longitudinal prospective human studies, but so far there is no clear conclusion about any protective effect of dietary or of supplementary antioxidants on diabetes risk.

The present study examined 1) weight change and fluctuation as risk factors for incident type 2 diabetes; 2) the association of baseline serum alpha-tocopherol or beta-carotene concentration and dietary intake of antioxidants with the risk of type 2 diabetes incidence; 3) the effect of supplementation with alpha-tocopherol or beta-carotene on the risk of incident type 2 diabetes; and on macrovascular complications and mortality among type 2 diabetics.

This investigation was part of the Alpha-Tocopherol, Beta-Carotene Cancer Prevention (ATBC) Study, a randomized, double-blind, placebo-controlled prevention trial, which has undertaken to examine the effect of alpha-tocopherol and beta-carotene supplementation on the development of lung cancer, other cancers, and cardiovascular diseases in male smokers aged 50-69 years at baseline. Men in their late middle age and who were smokers were randomly assigned to receive either 50 mg (50 IU) synthetic dl-alpha-tocopheryl acetate, 20mg synthetic beta-carotene, both, or placebo daily in a 2 x 2 factorial design experiment during 1985-1993. At study inclusion several background variables were assessed that covered medical history, weight, height, serum lipids, and blood pressure. At baseline the ATBC Study participants completed a detailed and validated food frequency questionnaire, and their serum alpha-tocopherol and beta-carotene concentrations were determined. Weight was measured once a year, every twelfth month, during the follow-up visits. Cases of incident diabetes were identified through a nationwide register of drug reimbursements of the Social Insurance Institution. As diabetic subjects at baseline were defined those who reported a history of physician-

diagnosed diabetes and those incident cases who had elevated fasting serum glucose (≥7.0 mmol/l) at baseline. Among those (n = 27 379) with no diabetes at baseline 305 new cases of type 2 diabetes were recognized during the intervention period and 705 during the whole follow-up to 12.5 years.

Among 20 952 participants, weight gain and weight fluctuation measured over a three year period were independent risk factors for 535 subsequent diabetes cases recorded for up to 9 years of follow-up. Multivariate adjusted relative risk (RR) was 1.77 (95% confidence interval [CI] 1.44-2.17) for weight gain of at least 4 kg compared to those with a weight change of less than 4 kg. The RR in the highest weight fluctuation quintile compared to the lowest was 1.64 (95% CI 1.24-2.17).

Dietary alpha-tocopherol or other tocopherols and tocotrienols as well as dietary carotenoids, flavonols, flavones and vitamin C were not associated with the risk of type 2 diabetes in 660 cases of incident diabetes reported during a median follow-up time of 10.2 years after multivariate adjustment. Baseline serum alpha-tocopherol and beta-carotene concentrations in the placebo group or in the whole study cohort were not associated with the risk of incident diabetes during follow-up of 12.5 years. Neither alpha-tocopherol nor beta-carotene supplementation affected the risk of diabetes during intervention or during a total follow-up at 12.5 years. The relative risks for participants who received alpha-tocopherol compared with nonrecipients and for participants who received beta-carotene compared with nonrecipients were 0.92 (95% CI 0.79-1.07) and 0.99 (95% CI 0.85-1.15), respectively. Furthermore, alpha-tocopherol or beta-carotene supplementation did not affect the risk of macrovascular complications or mortality of diabetic subjects during the 19 years follow-up time.

In conclusion, in this study of older middle-aged male smokers, weight gain and weight fluctuation were independent risk factors for type 2 diabetes. Intake of antioxidants, serum alpha-tocopherol or serum beta-carotene concentrations were not associated positively or inversely with the risk of type 2 diabetes. Supplementation with alpha-tocopherol or beta-carotene did not prevent type 2 diabetes. Neither did they prevent macrovascular complications, or mortality among diabetic subjects.

Keywords: epidemiology, alpha-tocopherol, beta-carotene, antioxidant, weight change, weight fluctuation, diabetic complications, mortality, randomized controlled trial, cohort study, type 2 diabetes

Tiivistelmä

Merja Kataja-Tuomola. Antioxidants, weight change and risk of type 2 diabetes. [Antioksidantit, painon muutos ja tyypin 2 diabeteksen riski]. Terveyden ja hyvinvoinnin laitos (THL). Tutkimus nro 59. 135 sivua. Helsinki, Finland 2011.
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Tyypin 2 diabeteksen ilmaantuvuus on kasvanut nopeasti maailmanlaajuisesti. Lihavuus on eräs tärkeimmistä tyypin 2 diabeteksen riskitekijöistä, joihin voidaan vaikuttaa: painon nousu lisää ja painon lasku vähentää riskiä. Painon vaihtelun vaikutus diabetesriskiin on kuitenkin epäselvä.

Reaktiiviset happiradikaalit ovat luultavasti osa insuliiniresistenssin kehittymisen ja haiman beetasolujen tuhoutumisen monimutkaista mekanismia. Antioksidanttien ja tyypin 2 diabeteksen kehittymisen yhteyttä on tutkittu useissa pitkittäisissä prospektiivisissa tutkimuksissa ihmisillä. Toistaiseksi selvää käsitystä ruokavalion tai ravintolisänä annettujen antioksidanttien vaikutuksesta tyypin 2 diabeteksen riskiin ei kuitenkaan ole.

Väitöskirjatyön tavoitteena oli tutkia: 1) painon muutoksen ja painon vaihtelun merkitystä tyypin 2 diabeteksen riskitekijöinä, 2) lähtötilanteen seerumin alfatokoferolin ja beetakaroteenin pitoisuuksien ja ravinnosta saatavien antioksidanttien yhteyttä tyypin 2 diabeteksen riskiin, 3) ravintolisänä annettujen alfatokoferolin ja beetakaroteenin vaikutusta tyypin 2 diabeteksen riskiin sekä näiden valmisteiden vaikutusta makrovaskulaarisiin komplikaatioihin ja kuolleisuuteen tyypin 2 diabeetikoilla.

Tutkimus tehtiin osana SETTI-tutkimusta (the Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study [ATBC]), joka on satunnaistettu, lumekontrolloitu kaksoissokkokoe, jonka tavoitteena oli selvittää, voidaanko keuhkosyöpää ja muita syöpiä ehkäistä beetakaroteeni- ja E-vitamiinivalmisteilla. Toissijaisena tavoitteena oli tutkia, mikä oli näiden valmisteiden vaikutus kuolleisuuteen sekä sydän- ja verisuonitautien ilmaantuvuuteen. Tutkittavilta kerätyt monipuoliset taustatiedot (sairaushistoria, paino, pituus, verenpaine) sekä laboratoriomääritykset (lähtötilanteen seerumin alfatokoferoli- ja beetakaroteenipitoisuudet), tekevät mahdollisiksi myös tutkimukset muiden kroonisten kansantautien, kuten tyypin 2 diabeteksen riskitekijöistä. Tutkimuksen kohderyhmänä olivat 50-69-vuotiaat tupakoivat miehet. Tutkimuksen osallistujat, 29 133 miestä, satunnaistettiin neljään yhtä suureen ryhmään, joista yksi ryhmä sai beetakaroteenia 20 mg, toinen alfatokoferolia 50 mg (50 IU), kolmas molempia ja neljäs lumevalmistetta päivittäin vuosina 1985-1993. Lähtötilanteessa tutkittavat täyttivät yksityiskohtaisen ja validoidun ruokavaliokyselyn. Paino mitattiin vuosittain seurantakäyntien yhteydessä. Diabetestapaukset kerät-

tiin Kansaneläkelaitoksen ylläpitämästä kansallisesta lääkekorvattavuusrekisteristä. Diabeetikoiksi lähtötilanteessa määriteltiin ne tutkittavat, jotka kertoivat lääkärin diagnosoineen heillä diabeteksen, sekä ne seurannan aikana ilmaantuneet uudet diabetestapaukset, joiden lähtötilanteen paastoseerumin sokeriarvo oli koholla (≥7,0 mmol/l). Lähtötilanteessa tyypin 2 diabetes oli 1700 tutkittavalla. Intervention aikana uusia diabetestapauksia ilmaantui 305 ja koko 12,5 vuoden seurannan aikana 705.

Painon nousu ja painon vaihtelu kolmen vuoden aikana olivat toisistaan riippumattomia diabeteksen riskitekijöitä. Ne, joiden paino nousi vähintään 4 kg, suhteellinen riski oli 1,77 (95 % luottamusväli 1,44-2,17) verrattuna niihin, joiden paino muuttui vähemmän kuin 4 kg. Kun painon vaihtelun ylintä viidennestä verrattiin alimpaan, riski oli 1,64 (95 % luottamusväli 1,24-2,17).

Ruokavalion alfatokoferoli tai muut tokoferolit ja tokotrienolit, karotenoidit, flavonolit ja flavonit kuten myöskään C-vitamiini eivät olleet yhteydessä tyypin 2 diabeteksen riskiin. Yhteyttä ei todettu myöskään lähtötilanteen seerumin alfatokoferoli- tai beetakaroteenipitoisuuden ja diabeteksen ilmaantuvuuden välillä. Ravintolisänä annetulla alfatokoferolilla tai beetakaroteenilla ei todettu olevan vaikutusta tyypin 2 diabeteksen riskiin intervention tai koko 12,5 vuoden seurannan aikana. Suhteellinen riski niillä, jotka saivat alfatokoferolivalmistetta oli 0,92 (95 % luottamusväli 0,79-1,07) verrattuna niihin, jotka eivät saaneet ja beetakaroteenia saaneilla 0,99 (95 % luottamusväli 0,85-1,15) verrattuna niihin, jotka eivät saaneet beetakaroteenia. Ravintolisänä annetulla alfatokoferolilla tai beetakaroteenilla ei myöskään ollut vaikutusta makrovaskulaaristen komplikaatioiden ilmaantumiseen tyypin 2 diabeetikoilla tai heidän kuolleisuuteensa 19 vuoden seuranta-aikana.

Väitöskirjatyön tulokset osoittavat, että painon nousu ja painon vaihtelu keski-ikäisillä ja vanhemmilla tupakoivilla miehillä olivat toisistaan riippumattomia tyypin 2 diabeteksen riskitekijöitä. Antioksidanttien saanti ravinnosta tai seerumin alfatokoferolin tai beetakaroteenin pitoisuudet eivät olleet yhteydessä tyypin 2 diabeteksen riskiin. Ravintolisänä annettu alfatokoferoli tai beetakaroteeni eivät estäneet uusia tyypin 2 diabetestapauksia eivätkä ne myöskään estäneet diabeteksen makrovaskulaarikomplikaatioita tai vähentäneet kuolleisuutta tyypin 2 diabeetikoilla.

Avainsanat: epidemiologia, alfatokoferoli, beetakaroteeni, antioksidantti, painon muutos, painon vaihtelu, diabeteksen komplikaatiot, kuolleisuus, satunnaistettu kontrolloitu tutkimus, kohorttitutkimus, tyypin 2 diabetes

Content

List of original publications

This thesis is based on the following original publications referred to in the text by their Roman numerals. Some unpublished data are also presented.

I Kataja-Tuomola M, Sundell J, Männistö S, Virtanen MJ, Kontto J, Albanes D, Virtamo J. Short-term weight change and fluctuation as risk factors for type 2 diabetes in Finnish male smokers. Eur J Epidemiol 2010;25:333-339.

II Kataja-Tuomola MK, Kontto JP, Männistö S, Albanes D, Virtamo J. Intake of antioxidants and risk of type 2 diabetes in a cohort of male smokers. Eur J Clin Nutr 2011 Jan 19; doi:10.1038/ejcn.2010.283

III Kataja-Tuomola M, Sundell JR, Männistö S, Virtanen MJ, Kontto J, Albanes D, Virtamo J. Effect of α-tocopherol and β-carotene supplementation on the incidence of type 2 diabetes. Diabetologia 2008;51:47-53.

IV Kataja-Tuomola MK, Kontto JP, Männistö S, Albanes D, Virtamo JR. Effect of alpha-tocopherol and beta-carotene supplementation on macrovascular complications and total mortality from diabetes: results of the ATBC Study. Ann Med 2010;42:178-186.

Abbreviations

ADA	American Diabetes Association
ANOVA	Analysis of variance
ATBC	Alpha-Tocopherol, Beta-Carotene Cancer Prevention
BMI	Body mass index
CI	Confidence interval
DECODE	The DECODE study. Diabetes epidemiology: collaborative analysis of diagnostic criteria in Europe
EPICNS	European Investigation of Cancer Norfolk Study
FFAs	Free fatty acids
FFQ	Food frequency questionnaire
FMCHES	Finnish Mobile Clinic Health Examination Survey
HDL	High-density lipoprotein
HOPE	Heart Outcomes Prevention and Evaluation
HPSCG	Heart Protection Study Collaborative Group
HR	Hazard ratio
ICD	International Classification of Diseases
IDR	Incidence density ratio
IRR	Incidence rate ratio
IQR	Interquartile range
MRC/BHF	Medical Research Council / British Heart Foundation
MRFIT	Multiple Risk Factor Intervention Trial
NHANES	National Health and Nutrition Examination Survey
NHEFS	National Health and Nutrition Examination Survey Epidemiologic Follow-up Study
NHS	Nurses' Health Study
OR	Odds ratio
PHS	Physicians Health Study
Q	Quintile
RH	Relative hazard
RMSE	Root-mean-square error
ROS	Reactive oxygen species
RR	Relative risk
SD	Standard deviation
SELECT	Selenium and Vitamin E Cancer Prevention Trial
SU.VI.MAX	Supplementation en Vitamines et Mineraux Antioxydants
USP	United States Pharmacopeia Unit
WHO	World Health Organization
WACS	Women's Antioxidant Cardiovascular Study
WHS	Women's Health Study

1 Introduction

It is estimated that by the year 2030 the prevalence of diabetes for all age-groups worldwide will be 4.4% (Wild et al. 2004). This percentage equates to 366 million people. The incidence of type 2 diabetes is increasing all over the world, but especially high numbers of estimated cases of diabetes will be found in India, China, U.S., Indonesia and Pakistan. In Finland, there are about 500 000 individuals with both types of diabetes: approximately 75% of them have type 2 diabetes (Diabetes 2009). During the 2004-2005 period 7.4% of men and 4.3% of women aged 45 to 74 had previously diagnosed diabetes (Peltonen et al. 2006). More than half of the patients with type 2 diabetes younger than 50 years of age were unaware of their disease in an urban population in Finland (The DECODE Study Group 2003).

Most often type 2 diabetes begins in adulthood (Narayan et al. 2003), and it is often accompanied by obesity, high blood pressure and disturbance of blood lipids (Laakso and Lehto 1998). The risk of type 2 diabetes increases with increasing values of body mass index (BMI) and with the duration of overweight and obesity (Wannamethee and Shaper 1999). There are inconsistencies in the findings concerning the association of weight fluctuation or cycling and risk for type 2 diabetes (Holbrook et al. 1989, Lissner et al. 1990, Morris and Rimm 1992, Hanson et al. 1995, French et al. 1997, Brancati et al. 1999, Field et al. 2004). Sedentary life style (Hu et al. 2001), unhealthy dietary habits (van Dam et al. 2002), smoking (Rimm et al. 1995, Mikhailidis et al. 1998), high levels of alcohol consumption (Wannamethee et al. 2002), age (Warram et al. 1997), ethnic group (Harris et al. 1998) and low birth weight (Hales et al. 1991, Eriksson et al. 2006) are other risk factors for type 2 diabetes.

The concordance of type 2 diabetes in monozygotic twins is approximately 60% compared with 20% in dizygotic twins (Newman et al. 1987, Kaprio et al. 1992). The consortia of several genome-wide association studies for type 2 diabetes have identified 19 common gene variants that increase the suspectibility to this disease (Lyssenko and Groop 2009).

Continuous diabetic hyperglycemia may predispose individuals to macrovascular (cardiovascular disease and peripheral vascular disease) (Pyörälä et al. 1987, Beckman et al. 2002) and microvascular (nephropathy, retinopathy, neuropathy) (Vinik and Vinik 2003) complications. Impaired glucose tolerance is itself associated with an increased risk of mortality (Balkau et al. 2004). Oxidative stress is one of several proposed mechanisms by which vascular endothelial function becomes impaired in diabetes (Guzik et al. 2002) and it has been proposed to be an

explanatory factor for the increased atherosclerosis seen in diabetes sufferers (Ceriello and Motz 2004).

The aim of this work was to study weight change and fluctuation as risk factors for incident type 2 diabetes and antioxidants in preventing incident type 2 diabetes and the complications of diabetes in a cohort of Finnish male smokers.

2 Review of literature

2.1 Type 2 diabetes

2.1.1 Definition

Diabetes is a condition with long-term increased blood glucose concentration. Type 2 diabetes is characterized by insulin resistance, but impaired insulin secretion also exists to a varying extent (Weyer et al. 1999, Lebovitz 2001a and 2001b). The diagnosis of type 2 diabetes is based on the measured blood glucose level. There is a direct relation between the risk of complications of diabetes and glycemia over time (Stratton et al. 2000)

The cut-off points for plasma glucose levels which are indicative of diabetes diagnosis are based on certain thresholds above which the incidence of diabetic vascular complications begin to increase (American Diabetes Association [ADA] 1997, Balkau 2000, Balkau et al. 2004), but these have changed over the years.

At the time when the Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study (ATBC) started in the 1980's, the diabetes diagnosis criteria were set at when the fasting plasma glucose concentration was ≥7.8 mmol/l, or when the two-hour plasma glucose concentration in an oral glucose tolerance test with 75 g glucose was 11.1 mmol/l or more (World Health Organization [WHO] 1985). A fasting plasma glucose concentration below 7.8 mmol/l and a 2 hour glucose tolerance test value between 7.8 mmol/l and 11.0 mmol/l indicated impaired glucose tolerance (WHO 1985).

In 1999 the World Health Organization (WHO) updated the concentration criteria as follows: in a person with no symptoms, the fasting blood plasma glucose concentration of at least 7.0 mmol/l, on two occasions, or plasma glucose ≥ 11.1 mmol/l in a 2-hour oral glucose test, was set to be the diagnostic threshold values for diabetes. Fasting plasma glucose concentration of less than 7.0 mmol/l, but 2-hour glucose tolerance test values of between 7.8 mmol/l and 11.1 mmol/l indicate impaired glucose tolerance. Impaired fasting glucose refers to a state for which fasting plasma glucose is slightly increased (6.1-6.9 mmol/l) and the 2-hour glucose tolerance test value is less than 7.8 mmol/l (WHO 1999).

2.1.2 Pathogenesis

A person is at risk of having diabetes if he or she has impaired glucose tolerance, impaired fasting glucose or in the case of females if she has had diabetes during pregnancy (Metzger et al. 1993). The development of type 2 diabetes results from the interaction between an individuals genetic background and environment (Gerich 1998). In type 2 diabetes impaired two-phasic pancreatic insulin secretion from beta-cells and decreased insulin action lead to elevated glucose levels. It has been estimated that approximately 50-60% of beta-cell insulin secretion capacity is lost by the time diabetes is clinically diagnosed (Butler et al. 2003). Hyperglycemia has been proposed to lead to an accumulation of a large number of reactive oxygen species in the beta-cells, with subsequent damage to the cellular components (Stumvoll et al. 2005).

Insulin resistance is a factor in which a given concentration of insulin is associated with a subnormal glucose response (Moller and Flier 1991). A few known genes are presumed to induce insulin resistance (Florez 2008). According to one hypothesis any perturbation that results in an accumulation of intracellular fatty acyl coenzyme As or other fatty acid metabolites in muscle and liver, either through increased delivery or decreased metabolism, might be expected to induce insulin resistance (Shulman 2000). Liver fat content has been shown to correlate significantly with fasting serum insulin concentrations when analysis was adjusted for insulin clearance (Spearman's nonparametric rank correlation coefficient, $r = 0.43$, $p<0.0001$) and with directly measured hepatic insulin sensitivity ($r = - 0.40$, $p = 0.0002$) (Kotronen et al. 2007).

Moreover, inherited or acquired defects in mitochondrial function that cause an alteration in the ability of muscle and liver to metabolize fatty acids, would also lead to an intracellular accumulation of fatty acid metabolites and subsequent defects in insulin signalling and action. Non-esterified fatty acids stimulate gluconeogenesis in the liver (Boden and Shulman 2002). Key enzymes of hepatic glucose production, such as phosphoenolpyruvatecarboxykinase and glucose-6-phosphatase, when overexpressed, have been shown to induce insulin resistance in vivo, and also disturbances of both glucose and lipid homeostasis (Postic et al. 2004).

Recently, it was stated that 19 genetic variants that are associated with an increase in the risk of type 2 diabetes are known (Groop and Lyssenko 2009). Most of them seem to influence the capacity of the beta-cells to increase insulin secretion. A hypothesis exists that hyperglycemia is the consequence of intrinsic beta-cell function deficiency rather than of a defect in the mechanism of compensation for insulin resistance (Mari et al. 2010).

2.1.3 Complications

Globally, type 2 diabetes is of considerable relevance because of its adherence to macro- and microvascular complications (Goldberg 1981, Laakso and Barrett-Connor 1989, Laakso et al. 1993). The risk for myocardial infarction in a diabetic person is as high as in a person with an earlier myocardial infarction (Schramm et al. 2008) and the risk of stroke for individuals in the 35-54 age group is 2.0 to 4.0 fold that of the general population (Folsom et al. 1999, Kissela et al. 2005).

Patients with diabetes more commonly develop the symptomatic forms of peripheral arterial disease and intermittent claudication than nondiabetic persons (Uusitupa et al. 1990). Diabetic nephropathy is the predominant cause of renal failure, and it accounts for nearly 44% of new cases (National Institute of Diabetes and Digestive and Kidney Diseases et al. 2007). Diabetic retinopathy is a leading cause of new-onset blindness in industrialized countries and an increasingly frequent cause of blindness in middle-income countries (Resnikoff et al. 2004). The WHO has estimated that diabetic retinopathy is responsible for 4.8% of the 37 million cases of blindness throughout the world (Resnikoff et al. 2004). The estimated prevalence of diabetic retinopathy and vision-threatening diabetic retinopathy was 28.5% and 4.4% among US adults with diabetes, respectively (Zhang et al. 2010). In Finland most of these complications are treated in hospital outpatient wards, and therefore the frequency of these complications cannot be determined on the basis of hospital discharge registers (Niemi and Winell 2006). Diabetic neuropathy, which is the most prevailing form of neuropathy worldwide, is the linchpin in diabetic foot ulcer (Boulton 2004, Boyko et al. 2006).

In a multicentre study the prevalence of neuropathy was 32.1% in type 2 diabetic patients in the United Kingdom hospital clinic population (Young et al. 1993). The annual mortality rate in a British study on 1694 diabetic patients was more than four times that of the mortality rate of the nondiabetic population, and cardiovascular disease accounted for almost one-half (49.1%) of all deaths in the diabetic population (Morgan et al. 2000).

2.2 Weight and type 2 diabetes

2.2.1 Mechanisms of the association

Studies reported in the 1980s and 1990s indicated that central obesity as estimated by waist hip ratio was predictive of the development of type 2 diabetes (Ohlson et al. 1988, Carey et al. 1997). Increased waist girth corresponds to increased amounts of visceral adipose tissue (Lemieux et al. 1996) and is, in turn, related to the development of type 2 diabetes (Alberti et al. 2005, Meisinger et al. 2006).

According to one hypothesis, visceral adipose tissue may have promoting influence on the development of insulin resistance by releasing free fatty acids, which drain directly into the liver and cause hepatic insulin resistance, and it is also suspected to contribute to the development of muscle insulin resistance (Boden and Shulman 2002). Weight loss studies have shown that the improvement in insulin sensitivity correlates with changes in visceral adipose tissue mass but not with total or subcutaneous adipose tissue mass (Goodpaster et al. 1999).

2.2.2 Epidemiological studies

2.2.2.1 Body mass index

Body mass index (BMI, kg/m^2) has been shown to be associated positively with type 2 diabetes in three large meta-analyses (Hartemink et al. 2006, Vazquez et al. 2007, Abdullah et al. 2010) and in one smaller meta-analysis (Guh et al. 2009) (Table 1). Vazquez et al. performed a meta-analysis on 32 studies selected from 432 publications. The publications were categorised in terms of different populations and being either normoglycemic or having impaired glucose tolerance at baseline with up to 25 years follow-up. All studies analyzed the progression from non-diabetes to diabetes. To assess the association between BMI and the incident diabetes rate, the pooled estimate across all studies for the relative risk was calculated. The pooled estimate of relative risk for incident diabetes per standard deviation increase for body mass index was 1.87 (95% CI 1.67-2.10) (Vazquez et al. 2007).

In another meta-analysis the selection of studies yielded 31 articles on epidemiologic studies of type 2 diabetes as a function of body mass index. It showed that the pooled relative risk for type 2 diabetes was 1.18 (95% CI 1.16-1.20) per unit of increasing body mass index, although there was a clear heterogeneity of studies (Hartemink et al. 2006) (Table 1).

In a meta-analysis of 7 studies with North American and European populations, elevated BMI was significantly associated with type 2 diabetes in men and women (Guh et al. 2009) (Table 1). The association between being overweight as defined by BMI (BMI $\geq$ 25 kg/m^2) and the incidence of type 2 diabetes was stronger in females: RR was 3.92 (95% CI 3.10-4.97) when overweight women were compared to those with normal weight. In overweight men the relative risk for incident type 2 diabetes was lower 2.40 (95% CI 2.12-2.72) compared to men with normal weight. Obesity (BMI $\geq$30 kg/m^2) was most strongly associated with the incidence of type 2 diabetes in women (RR 12.4; 95% CI 9.0-17.1).

One objective in the most recent meta-analysis was to examine the magnitude of the risks for developing type 2 diabetes for overweight and obese populations and compare them with those individuals of normal weight. That meta-analysis study

combined 16 prospective cohort studies and 2 nested case control studies with North American, European and Asia-Pacific origin populations. Overweight was associated with a 3 fold higher risk for diabetes compared to those with normal weight (RR 2.99; 95% CI 2.42-3.72). Obese individuals were associated with a 7 fold higher risk of diabetes compared to those with normal weight (RR 7.19; 95% CI 5.74-9.00) (Abdullah et al. 2010) (Table 1).

The results of a recently published follow-up study of the relation of incident type 2 diabetes to weight patterns during middle age in a subset of the Framingham Heart Study were in line with previous studies. In 1476 adults, 217 cases of type 2 diabetes were diagnosed. Overall weight status observed between 40 to 50 years of age was strongly associated with the development of type 2 diabetes. Multivariate adjusted hazard ratio (HR) was 2.90 (95% CI 2.0-4.10) for overweight participants compared to those with normal weight, and multivariate adjusted HR for obese participants was 7.70 (95% CI 4.90-12.10) (Waring et al. 2010).

Table 1. Summary of the meta-analyses of prospective cohort studies or nested case-control studies for the association between BMI and the risk of type 2 diabetes.

Reference	Studies	Sample size of the cohorts	Review period/ follow-up years	Risk for type 2 diabetes		95% Confidence interval
Hartemink et al. 2006	31 cohort studies women, men EU, U.S.	766–46 634	1980–2004 / 4–25	RR / BMI unit increase	1.18	1.16 – 1.20
Vazquez et al. 2007	31 cohort studies and 1 nested case control study women, men EU, U.S., Canada, Korea, Jamaica, Taiwan, Mexico, Japan	72–31 702	1966–2004 / 2−25	RR / SD increase in BMI	1.87	1.67 – 2.10
Guh et al. 2009	7 studies women, men U.S., Germany, UK	Men 3055–22 172 Women 2957–84 941	Review period not shown/ 3.7–23.8 for men, 6.9–16 for women	Pooled RR for men: overweight vs. normal obese vs. normal for women: overweight vs. normal obese vs. normal	 2.40 6.74 3.92 12.41	 2.12 – 2.72 5.55 – 8.19 3.10 – 4.97 9.03 –17.06
Abdullah et al. 2010	16 cohort studies and 2 nested case-control studies women, men U.S., Asia-Pacific, Europe	2902–154 989	1966–2008 / 2−27	Pooled RR overweight vs. normal obese vs. normal	 2.99 7.19	 2.42 – 3.72 5.74 − 9.00

RR, relative risk; SD, standard deviation

2.2.2.2 Weight gain

Of the 13 prospective follow-up studies shown in Table 2, nine have reported increased risk of type 2 diabetes with weight gain, and most of them have also demonstrated a higher risk with higher body weight gains (Holbrook et al. 1989, Colditz et al. 1995, Hanson et al. 1995, Ford et al. 1997, Resnick et al. 2000, Koh-Banerjee et al. 2004, Oguma et al. 2005, Wannamethee et al. 2005, Jacobs-van der Bruggen et al. 2010). Estimation of weight, time range for weight change and follow-up period have largely varied between different studies. Most of the studies assessed the change in body weight, two studies used change in body mass index (Oguma et al. 2005, Waring et al. 2010). With a few exceptions the studies originate from the United States and they include results from large prospective American cohorts.

In a large prospective follow-up study, the Nurses' Health Study (NHS) with up to 114 281 middle-aged women, those who had gained at least 20 kg of weight in adulthood were found to have a 12 fold risk for clinical diabetes than those with loss or gain of weight of under 5 kg (Colditz et al. 1995) (Table 2). However, in the National Health and Nutrition Examination Survey Epidemiologic Follow-up Study (NHEFS) the risk for diabetes was much lower (RR 3.84; 95% CI 2.04-7.22) for those who gained ≥20 kg of weight (Ford et al. 1997). In a subsample of 1929 adults in the same study aged 25 to 74 years who at baseline were already overweight, one kg of weight gained annually over 10 years was associated with a 49% increase in risk of developing diabetes in the subsequent 10 years (Resnick et al. 2000). The association between weight gain and risk of incident type 2 diabetes is well-established in long-term prospective follow-up studies (Holbrook et al. 1989, Colditz et al. 1995, Oguma et al. 2005, Wannamethee et al. 2005). Nonetheless, there is a discrepancy between the above mentioned results and the result of a recent substudy of the Framingham Heart Study: weight gain compared to stable weight during middle age was not associated with incident diabetes in 1476 participants during follow-up to 25 years (multivariate adjusted HR 1.20; 95% CI 0.80-1.70) (Waring et al. 2010). Far less is known about short-term weight change and risk of incident type 2 diabetes, the results of few prospective follow-up studies are inconsistent (Ishikawa-Takata et al. 2002, Mishra et al. 2007, Jacobs-van der Bruggen et al. 2010). Among Dutch adults 5-year weight gain during subsequent follow-up of five years was associated with an increased risk of type 2 diabetes when adjusted for initial BMI. However, no significant association was found if the association was adjusted for attained BMI (Jacobs-van der Bruggen et al. 2010).

2.2.2.3 Weight loss

Weight loss has been shown to reduce the risk of developing type 2 diabetes in the Finnish Diabetes Prevention Study with 522 participants (Tuomilehto et al. 2001). Similar findings have been obtained in U.S. in the Multiple Risk Factor Intervention Trial (MRFIT) with 11 827 participants and in the Diabetes Prevention Program with 1079 participants (Davey

Smith et al. 2005, Hamman et al. 2006). These three studies were intervention trials with a mean of 3.2 follow-up years for Diabetes Prevention Study and for Diabetes Prevention Program and up to 7 years follow-up for the MRFIT study. A weight loss of between 3-5 kg was associated with a reduced risk developing diabetes when compared with the weight stable group. The hazard ratio (HR) was 0.40 (95% CI 0.30-0.70 $p<0.001$) in the Diabetes Prevention Study and 0.42 (95% CI 0.35-0.51 $p<0.0001$) in the Diabetes Prevention Program. In the MRFIT study the decrease in BMI of 1kg/m^2 in the intervention group reduced the risk for incident type 2 diabetes by 25% (HR 0.75; 95% CI 0.70-0.81) and in the usual care group by 16% (HR 0.84; 95% CI 0.78-0.90) in non-smokers. In smokers with this BMI decrease the risk for incident type 2 diabetes reduced by 17% in the intervention group (HR 0.83 95% CI 0.79-0.88) and by 19% in the usual care group (HR 0.81; 95% CI 0.77-0.86).

Large long-term prospective follow-up studies have shown weight loss to be inversely associated with the risk of incident diabetes (Colditz et al. 1995, Will et al. 2002, Wannamethee et al. 2005) (Table 2). In the Nurses' Health Study, type 2 diabetes risk was approximately halved by a weight decrease of between 5-10 kg and among those with at least 20 kg weight decrease the diabetes risk was diminished by nearly 90% (Colditz et al. 1995). Among adult U.S. men and women self-reported intentional weight loss was associated with a lower diabetes risk, which was gradually decreased by increased weight loss (Will et al. 2002). In the British Regional Heart Study on 6194 participants, weight loss reduced the risk of developing diabetes when compared with those whose weight was stable; the relative risk was 0.62 (95% CI 0.42-0.90) for those who lost more than 4% of their weight during 5 years (Wannamethee et al. 2005) (Table 2). Significant decreased risk of type 2 diabetes during follow-up of four years was also demonstrated among the Health Professional Study participants with a weight loss of at least 6 kg over 10 years (Koh-Banerjee et al. 2004). In the NHEFS cohort, one kg weight loss annually over 10 years was associated with a 33% lower risk of diabetes among those who were initially overweight (Resnick et al. 2000). Nevertheless, in a previous analysis of the whole NHEFS cohort with 8545 adults participants with weight loss were found to have no reduction in the risk of developing diabetes when compared with those whose weight was stable (Ford et al. 1997). Similarly, several other prospective studies reported non-significant associations between weight loss and type 2 diabetes risk (Ishikawa-Takata et al. 2002, Oguma et al. 2005, Mishra et al. 2007, Jacobs-van der Bruggen et al. 2010, Waring et al. 2010). In contrast, a weight loss of 4.5 kg compared to a stable weight increased the risk of type 2 diabetes in 2000 U.S. participants aged 50 years or over (RR 1.70, $p<0.05$) (Holbrook et al. 1989). Accordingly, there are clear discrepancies in the results of the prospective follow-up studies of weight loss and risk of incident type 2 diabetes.

2.2.2.4 Weight fluctuation

The association of obesity and weight increase with risk of type 2 diabetes is well established, but the association between weight fluctuation and risk of type 2 diabetes is unclear. Among middle aged U.S. women fluctuation of self-reported weight determined as an index of standard error of the estimate (i.e. the slope of the regression line describing weight as a function of age) was associated with increased risk of type 2 diabetes in a retrospective study (Morris and Rimm 1992). A summary of the prospective studies are shown in Table 3. In the Iowa Women's Health Study with up to 914 cases of incident diabetes, large weight cycling was positively associated with the risk of diabetes (RR 1.70; 95% CI 1.25-2.29) when compared with combined stable weight plus small weight gain categories (French et al. 1997). Small weight cycling was not associated with a risk of diabetes in the same study (RR 1.38; 95% CI 0.94-2.03). High BMI variability between 20 to 49 years of age in former medical male students in the U.S. was associated with an increased risk of diabetes (Brancati et al. 1999). The risk was doubled in the highest BMI variability quartile compared to the others (Brancati et al. 1999).

Conversely, neither those women with severe weight cycles (severe cyclers), nor mild weight cycles (mild cyclers) had an increased risk for incident diabetes in the Nurses' Health Study during a six year follow-up (Field et al. 2004) (Table 3). Women were classified as severe weight cyclers, if they had intentionally lost weight ≥9.1 kg, at least three times over the previous 4 years. Women who had intentionally lost ≥ 4.5 kg three or more times, but did not meet the criteria for severe weight cyclers, were classified as mild weight cyclers. In a study on Pima Indians (383 women and 201 men), there was no association found between weight fluctuation and incident diabetes (Hanson et al. 1995). In a recently published follow-up study, a subset of the Framingham Heart Study, cycling of BMI 1 kg/m^2 or more during middle age increased the risk for incident diabetes compared to non-cycling weight (hazard ratio 1.60; 95% CI 1.20–2.10) (Waring et al. 2010). However, after adjustment for overall weight status, weight cycling was no longer associated with incident diabetes (hazard ratio 1.10; 95% CI 0.80-1.50).

Table 2. Prospective follow-up studies of the association between weight gain or weight loss and the risk of type 2 diabetes.

Reference	Study population/ follow-up time	Cases	Weight measurement, time range for weight change	Reference weight, kg	Weight gain, kg	RR (95% CI)	Weight loss, kg	RR (95% CI)
Holbrook et al. 1989	U.S., 1114 women 886 men aged ≥50 years/ 15 years	142 women 142 men	Weight history interview, weight change between ages 40-60 years	No loss, no gain	4.5	1.90[a]	4.5	1.70[a]
Colditz et al. 1995	U.S., 114 281 women aged 30-55 years/ 14 years	2204	Self-reported at 18 years and at baseline	Loss/gain 0-4.9	5.0-7.9 8.0-10.9 11.0-19.9 ≥ 20	1.9[b] (1.5-2.3) 2.7 (2.1-3.3) 5.5 (4.7-6.3) 12.3 (10.9-13.8)	5.0-10.9 11.0-19.9 ≥20	0.54[b] (0.4-0.8) 0.23 (0.1-0.4) 0.13 (0.1-0.3)
Hanson et al. 1995	U.S., 906 women 552 men aged ≥20 years/ 7.4 years (women), 7.2 years (men)	306 women 155 men	Weight measured at least 2 years apart	0 kg/year	2.7 kg/year 2.6 kg/year	IRR 0.98[c] (0.87-1.11) 1.24 (1.04-1.49)		
Ford et al. 1997	U.S., 8545 women, men, aged ≥25 years/ 8-10 years	297 women 190 men	Weight measured in 1971-1975 and 1982-1984	Loss/gain 0-4.9	5-<8 8-<11 11-<20 ≥20	HR 2.11[d] (1.40-3.18) 1.19 (0.75-1.89) 2.66 (1.84-3.85) 3.84 (2.04-7.22)	5-<11 ≥ 11	HR 1.13[d] (0.72-1.80) 0.80 (0.46-1.40)
Resnick et al. 2000	U.S., 1929 women, men aged 25-74 years/ 10 years	251	Weight measured approximately 10 years apart	0 kg/year	0.1kg/year 0.5 1.0 1.5 2.0	OR 1.04[e] (1.03-1.06) 1.22 (1.13-1.31) 1.49 (1.29-1.73) 1.82 (1.46-2.27) 2.22 (1.66-2.98)	0.1kg/year 0.5 1.0 1.5 2.0	OR 0.96[e] (0.95-0.97) 0.82 (0.76-0.88) 0.67 (0.58-0.78) 0.55 (0.44-0.68) 0.45 (0.34-0.60)
Ishikawa-Takata et al. 2002	Japan, 4385 men aged 18-59 years/ 4 years	242	Weight measured annually	Loss/gain 0-2	>2	1.14[f] (0.85-1.54)	>2	1.16[f] (0.80-1.69)
Will et al. 2002	U.S., 111 285 women 101 285 men aged ≥ 30 years/ 13 years	5658 women	Self-reported (intentional weight loss) at baseline	No change			0.1-9.0 9.1-18.1 18.2-27.1 27.2-36.2 ≥36.3	IDR 0.76[g] (0.70-0.84) 0.72 (0.65-0.78) 0.66 (0.57-0.77) 0.47 (0.34-0.66) 0.36 (0.21-0.60)

Will et al (continued)		4669 men					0.1-9.0 9.1-18.1 18.2-27.1 27.2-36.2 ≥36.3	0.85[g] (0.76-0.95) 0.77 (0.68-0.86) 0.74 (0.59-0.93) 0.31 (0.16-0.60) 0.36 (0.13-0.98)
Koh-Banerjee et al. 2004	U.S., 22 171 men aged 40-75 years/ 4 years	305	Self-reported biannually, weight change over 10 years	Loss/gain 0-2	3-5 6-8 ≥9	1.4[h] (1.0-1.9) 1.6 (1.1-2.4) 2.1 (1.5-3.0)	3-5 ≥6	1.0[h] (0.7-1.5) 0.5 (0.3-0.9)
Oguma et al. 2005	U.S., 20 187 men mean age 45.9 years/ up to 36 years	1223	Weight measured at mean age of 18.5 years, self-reported at mean age of 45.9 years	BMI change per decade ± 0.5	BMI change per decade >0.5-1.0 >1.0-1.5 >1.5-2.0 >2.0-3.0 >3.0	 1.13[i] (0.92-1.40) 1.69 (1.38-2.06) 2.08 (1.69-2.56) 3.45 (2.81-4.23) 5.34 (4.13-6.89)	BMI change per decade ≤ -0.5	 1.30[i] (0.87-1.92)
Wannamethee et al. 2005	UK., 6194 men aged 40-59 years/ 15 years	327	Weight measured and self-reported 5 years later	Loss/gain < 4%	4-10% >10%	1.26[j] (0.97-1.64) 1.76 (1.16-2.67)	> 4%	0.62[j] (0.42-0.90)
Mishra et al. 2007	Australia, 7239 women aged 45-50 years/ 3 years	207	Self-reported, weight change over 3 years	Loss/gain per year <1.5%	1.5 to < 2.5% 2.5-5.0% >5%	OR 1.24[k](0.82-1.87) 0.93 (0.60-1.42) 1.54 (0.92-2.56)	1.5 to <2.5% 2.5-5.0% >5.0%	OR 0.63[k](0.32-1.21) 1.34 (0.82-2.14) 0.56 (0.22-1.41)
Jacobs-van der Bruggen et al. 2010	The Netherlands, 7837 women, men aged 20-59 years/ 5 years	124	Weight measured, weight change over 5 years	Loss/gain ± 2.0	2.0-4.0 4.0-6.0 >6.0 2.0-4.0 4.0-6.0 >6.0	OR 0.9[l] (0.5-1.6) 1.3 (0.8-2.3) 2.4 (1.4-4.0) OR 0.7[m](0.4-1.2) 0.8 (0.5-1.4) 1.0 (0.6-1.7)	>2.0 >2.0	OR 0.7[l](0.4-1.3) OR 1.1[m](0.6-2.1)
Waring et al. 2010	U.S., 1474 women, men aged 28-40 years / up to 25 years	217	Weight measured biannually, weight change between ages of 40-50 years	BMI change ± 0.6 (IQR)	BMI change 0.6 to 2.4 (IQR)	HR 1.2[n] (0.8-1.7)	BMI change -0.6 to -2.1 (IQR)	HR 1.1[n] (0.7-1.8)

Table 2 (continued)

RR, relative risk; CI, confidence interval; IRR, Incidence rate ratio; HR, hazard ratio; OR, odds ratio; IDR, incidence density ratio; BMI, body mass index; IQR, interquartile range.

Multivariate analyses are adjusted for:

[a] age, sex, current body mass index and current smoking status, and weight and dieting variables listed.

[b] age and body mass index at age 18 years.

[c] age and BMI (women), smoking (men).

[d] age, age^2, sex, race, education, education^2, smoking status, cholesterol, cholesterol^2, systolic blood pressure, systolic blood pressure^2, antihypertensive medication, baseline body mass index, and alcohol consumption.

[e] age, age^2, BMI, sex, race, skinfold ratio and systolic blood pressure.

[f] age, BMI at 1994, smoking, alcohol intake, family history, and baseline value of systolic blood pressure, fasting blood glucose, or total cholesterol.

[g] age, prebaseline BMI, race, educational level, dietary intakes of fat and carbohydrates, alcohol use, smoking frequency, exercise level, history of heart disease, stroke, hypertension, cancer or cirrhosis, symptoms including pain in chest, shortness of breath, fatigue, loss of appetite, blood in stool, or blood in urine, and general health status.

[h] smoking status, physical activity, family history, dietary fiber, and body mass index in 1986.

[i] age, physical activity, smoking, hypertension, and family history of diabetes.

[j] age,social class, smoking, physical activity, alcohol intake, antihypertensive treatment, undiagnosed coronary heart disease, forced expiratory volume in 1 second (FEV_1), systolic blood pressure, total cholesterol, and initial BMI.

[k] age,BMI, physical activity, smoking status, education, menopause status, area of residence.

[l] age, agexage, gender, initial BMI, initial hypertension, and initial total/high density lipoprotein cholesterol ratio.

[m] age, agexage, gender, attained BMI, atteined hypertension, and 5-year change in total/high density lipoprotein cholesterol ratio.

[n] weight status at age 25 years, gender, ever use of hormones (women), alcohol consumption, smoking, education, overall weight status and weight cycling.

Table 3. Prospective follow-up studies of the association between weight fluctuation and the risk of type 2 diabetes.

Reference	Country, study population/ follow-up time	Cases	Weight measurement	Determination of weight fluctuation	Weight fluctuation categories	RR (95% CI)
Hanson et al. 1995	U.S., 383 women 201 men, aged ≥ 20 years/median 6.3 years	162	Weight measured approximately 2 years apart	The root-mean-square error (RMSE) of the slope of the regression line of weight with time over approximately 6 years	75th percentile of RMSE (4.9 kg) vs. 25th percentile (2.0 kg)	IRR 1.03[a] (0.85-1.25)
French et al. 1997	U.S., 33 834 women 55-69 years/ 6 years	914	Recalled weights at ages 18, 30, 40 and 50 years	Weight change between any two adjacent ages (at 30 and 40 years) - large cycle: weight gain ≥10% of weight and weight loss ≥10% of weight during different intervals, - small cycle: weight gain >5% of weight and weight loss >5% of weight during different intervals.	Large cycle vs. stable weight + small gain[a] Small cycle vs. stable weight + small gain[a]	1.70[b] (1.25-2.29) 1.38[b] (0.94-2.03)
Brancati et al. 1999	U.S., 916 men aged 50 years/ mean 15.6 years	35	Weight measured at 20 to 29 years, self-reported thereafter every 3 to 5 years up to 49 years of age	Sum of squared distances between the reported BMI and the BMI predicted from the random-effects model at the same age, divided by the number of reported BMI values during 20 to 49 years.	Highest BMI variability quartile vs. other quartiles	2.10[c] (1.00-4.60)
Field et al. 2004	U.S., 46 634 women aged 25-43 years/ 6 years	418	Self-reported intentional weight loss over past 4 years at baseline	Intentional weight loss over the previous four years - severe cycler: ≥9.1 kg three or four times - mild cycler: ≥4.5 kg three or more times, but not severe cycle - non-cycler: person who did not meet the criteria described above.	Severe cycler vs. non-cycler Mild cycler vs. non-cycler	1.39[d] (0.90-2.13) 1.11[d] (0.80-1.37)
Waring et al. 2010	U.S., 1476 women, men, 28-40 years/ mean 24 years	217	Weight measured biannually	Weight cycling was determined by principal component analysis of BMI during middle age (from 40 to 50 years)	Cycling of BMI 1kg/m^2 or more vs. no cycling	HR 1.1[e] (0.8-1.5)

RR, relative risk; CI, confidence interval; RMSE, root-mean-square error; IRR, incidence rate ratio; HR, hazard ratio
Multivariate analyses are adjusted for:
[a]age, sex, BMI, smoking, rate of weight gain, the time between the initial and referent examinations.
[b]baseline age, waist/hip ratio, BMI, BMI^2 smoking status, pack years of cigarettes, education, physical activity, alcohol, marital status, hormone replacement.
[c]age at enrollment, BMI at age 25, physical activity level at enrollment, maternal history of diabetes, time-dependent smoking,
[d]age, BMI, smoking, family history of diabetes, hours per week of vigorous activity, hours per week of sitting, alcohol intake, magnesium intake and total calories.
[e]weight status at age 25 years, gender, ever use of hormones (women), alcohol consumption, smoking, education, overall weight status, weight changes during middle age.

2.3 Antioxidants and type 2 diabetes

2.3.1 Biology and sources of antioxidants

Nonenzymatic dietary antioxidants include *inter alia* vitamins E and C and carotenoids (Johansen et al. 2005). Vitamin E is a lipid-soluble, plant-origin vitamin that consists of eight structurally related compounds, which react directly with peroxyl and superoxide radicals and singlet oxygen moieties, and in so doing protect membranes from lipid peroxidation (Maritim et al. 2003, Hensley et al. 2004).

Alpha-tocopherol is the most active form of vitamin E in humans (Johansen et al. 2005), and these terms are used synonymously in this thesis. One United States Pharmacopeia (USP) unit of vitamin E has the activity of 1mg of all rac-alpha-tocopheryl acetate (formerly dl-alpha-tocopheryl acetate) or 0.74 mg of natural (RRR-) alpha-tocopheryl acetate (Institute of Medicine 2000). In human nutrition, the richest sources of vitamin E are vegetable oils, unprocessed cereal grains and nuts (National Institute for Health and Welfare. Nutrition Unit 2010).

All carotenoids possess a polyisopropenoid structure (Britton 1995). They are natural pigments synthesized by plants and micro organisms, but not by animals, and classified into carotenoid hydrocarbons (known as carotenes) and oxygenated carotenoids (known as xanthophylls) according to their chemical composition (Paiva and Russell 1999). Among 600 carotenoids approximately 50 have vitamin A activity (Bendich and Olson 1989). A wide range of carotenoids have been identified in human plasma and tissues including: cyclic (beta-carotene, alpha-carotene), acyclic carotenes (lycopene, phytoene) together with a number of xanthophylls (zeaxanthin, lutein and beta-cryptoxanthin) (Khachik et al. 1997). Good sources of carotenoids are carrots and colourful vegetables (National Institute for Health and Welfare. Nutrition Unit 2010).

Vitamin C (ascorbic acid) is a non-enzymatic, water-soluble six-carbon lactone, that scavenges (reduces a reactive free radical by the formation of a less reactive compound) superoxide, peroxyl, hydroxyl, and sulphur radicals and nitrogen-oxygen radicals (Clark 2002, Padayatty et al. 2003) and in combination with vitamin E ascorbic acid inhibits hydroxyperoxide formation (Maritim et al. 2003). Citrus fruits and vegetables are the best sources of vitamin C (Clark 2002).

Over 8000 phenolic and polyphenolic compounds are produced by plants (Duthie and Crozier 2000). Flavonoids represent important polyphenols in the human diet and can be divided into several classes according to the degree of oxidation of the oxygen heterocycle (flavonols, flavones, flavanols, flavanones and anthocyanidins)

(Tapiero et al. 2002, Prior 2003). In human nutrition dietary sources of flavonoids and phenolic acids include: tea and red wine as catechins, citrus fruits as flavanones, onions, olives, apples, cultivated and wild berries in the form of flavonols, such as quercetin, cherries, strawberries, grapes, colored fruits for anthocyanides and coffee for caffeic acid (Hertog et al. 1992 and 1993, Croft 1998, Häkkinen et al. 1999). However, large differences exist in the content of these compounds that depend upon the cultivar (Herrmann 1988), and also upon growing conditions. There are indications for positive effects on glucose homeostasis exerted by polyphenols and polyphenol-rich plant extracts in *in vitro* and animal studies, and epidemiological evidence supports beneficial effects of polyphenol- rich diets (Hanhineva et al. 2010). Dietary polyphenolic compounds may influence glucose metabolism by several different mechanisms, such as the inhibition of carbohydrate digestion and glucose absorption in the intestine, stimulation of insulin secretion from the pancreatic beta-cells, modulation of glucose release from the liver, activation of insulin receptors, alteration of glucose uptake by the insulin-sensitive tissues, modulation of hepatic glucose output (Hanhineva et al. 2010).

2.3.2 Free radicals and type 2 diabetes and its complications

Free radicals are atoms or molecules that have one or more unpaired electrons in their atomic structure and are thus generally highly reactive. The term is also used of other reactive species that lead to or result from free radical reactions (Devasagayam et al. 2004). Oxygen can give rise to highly reactive oxygen species (ROS), but also nonoxygen reactive species do exist, such as nitrogen and chlorine species (Rosen et al. 2001).

Overproduction of ROS results in oxidative stress, a process that can be an important mediator of cell structure damage, including lipids and membranes, proteins and DNA (Valko et al. 2007). Oxidative stress has been defined as a serious imbalance between the production of reactive species and antioxidant defences (Halliwell 1995, Turrens 2003) and it may play a role in the development of insulin resistance (Fridlyand and Philipson 2006). Mitochondria are known to be the main source of ROS (Turrens 2003). In diabetes, hyperglycemia is one cause of enhanced free radical production (Brownlee 2001). Fatty acid oxidation can increase ROS production (Fridlyand and Philipson 2006), but free radicals are also produced as by-products of normal aerobic metabolism (Clark 2002), and concentrations of free radicals in the body may also be increased by environmental factors such as cigarette smoke (Fang et al. 2002, Devasagayam et al. 2004).

Beta-cells are particularly susceptible to oxidative stress (Maechler et al. 1999). In type 2 diabetes, excessive ROS could promote the inhibition of insulin synthesis (Evans et al. 2002, Robertson et al. 2003, Robertson and Harmon 2007). It has been

confirmed from *in vivo* studies that high glucose concentrations induce mitochondrial ROS, which suppresses the first phase insulin secretion (Sakai et al. 2003). Increased plasma concentration of free fatty acid (FFA) that induces intramyocellular lipid accumulation in humans has been proposed to play a critical role in pancreatic beta-cell death (McGarry 2002, Azevedo-Martins et al. 2006). The FFA and high glucose induce inflammation through oxidative stress whereas antioxidants have been shown to reverse this phenomen (Esposito et al. 2002, Newsholme et al. 2007).

Diet-derived vitamins C and E detoxify free radicals directly. However, under some conditions these vitamins foster toxicity by producing pro-oxidants (Maritim et al. 2003). Antioxidants have been able to prevent the destruction of beta-cells by inhibiting the peroxidation chain reaction (Slonim et al. 1983, Murthy et al. 1992). Beta-carotene is a precursor of vitamin A and seems to act as a lipid antioxidant by singlet oxygen quenching in vitro (Paiva and Russell 1999). Flavonoids can act as antioxidants through a number of pathways: by free radical scavenging (Croft 1998), by their metal chelating properties, by their effects on cell signalling pathways and by their effects on gene expression (Soobrattee et al. 2005).

Free radicals are also hypothesized to be involved in the development of the complications of diabetes. Hyperglycemia leads to an increased production of ROS by the non-enzymatic glycoxidation of proteins (Jakus and Rietbrock 2004). The advanced glycation end products increase vascular permeability and procoagulant activity and are suggested to contribute significantly to the multiorgan complications of patients with diabetes (Bucala et al. 1994).

2.3.3 Epidemiological studies of fruits, vegetables and antioxidants and risk of type 2 diabetes

2.3.3.1 Fruits and vegetables

The results of the prospective follow-up studies on the effects of fruit and vegetable intake on the risk of diabetes or disturbance in glucose metabolism are contradictory. In two prospective cohort studies the National Health and Nutrition Examination Survey (NHANES) I Epidemiologic Follow-up Study, and the Finnish Mobile Clinic Health Examination Survey higher intake of fruits, vegetables and berries was inversely associated with the risk of diabetes (Ford and Mokdad 2001, Montonen et al. 2005). Among 5207 women in the NHANES I Epidemiologic Follow-up Study in the USA with a follow-up of up to 20 years the relative risk for those who consumed five or more servings of fruit and vegetables per day was 0.61 (95% CI 0.42-0.88) compared to those who consumed none (Ford and Mokdad 2001). A limitation of the study was the use of a single 24-h dietary recall. The total energy

intake was not included in the adjustments either. In a Finnish study which investigated a total of 4304 participants aged 40-69 years and followed-up for 23 years, 383 incident cases of diabetes were diagnosed (Montonen et al. 2005). The relative risks of developing type 2 diabetes between the extreme quartiles of the intakes were 0.69 (95% CI 0.50-0.93; p = 0.02 trend) for green vegetables and 0.69 (95% CI 0.51-0.92; p = 0.03 trend) for fruit and berries. In a cohort study of 338 elderly Finnish and Dutch males followed for 30 years, an inverse association between higher consumption of vegetables and legumes and impaired glucose tolerance (2-h postload glucose concentration) was found (Feskens et al. 1995). However, in the Nurses' Health Study, which was a large follow-up study on 4529 cases of incident diabetes with 18 years of follow-up, no association between fruit and vegetable intake and the risk of diabetes was detected in age-adjusted or multivariate models (Bazzano et al. 2008). Neither was a protective association found in two other follow-up studies (Iowa Women's Health Study and Women's Health Study) between the intake of fruit and vegetables and risk of incident type 2 diabetes (Meyer et al. 2000, Liu et al. 2004).

2.3.3.2 Prospective studies on antioxidants

In a meta-analysis of antioxidant intake and the risk of diabetes on up to 139 793 participants and 8813 incident cases of type 2 diabetes with a mean follow-up of 13 years, the pooled relative risk of type 2 diabetes was 0.87 (95% CI 0.79-0.98; p = 0.02) for the highest compared with the lowest estimated antioxidant levels (Hamer and Chida 2007). A significant heterogeneity between the studies was displayed, though (p = 0.01). In subgroup analysis, a significant protective effect was demonstrated for vitamin E (RR 0.56; 95% CI 0.35-0.88; p = 0.01), and for total carotenoids (RR 0.76; 95% CI 0.58-0.99; p = 0.04). This meta-analysis included nine prospective cohorts with healthy participants at baseline. Antioxidant status was assessed by a range of methods, including plasma and serum levels, and estimated dietary intake. There was heterogeneity for the associations of vitamin E, which was attributed to the different types of vitamin E measures (p = 0.04).

When vitamin E and carotenoid intake and the risk of incident diabetes were studied in the Finnish Mobile Clinic Health Examination Survey, an inverse association was found between the intake of vitamin E and beta-cryptoxanthin and the risk of incident diabetes (p = 0.02 trend over quartiles of vitamin E, and p<0.001 trend over beta-cryptoxanthin quartiles) (Montonen et al. 2004, Table 4). However, the majority of studies that have examined antioxidant intake and the risk of diabetes have shown no significant association (Table 4). The largest studies with up to 38 018 participants were drawn from cohorts of U.S. participants (Song et al. 2005, Nettleton et al. 2006, Wang et al. 2006a). In two Finnish studies (Knekt et al. 2002, Montonen et al. 2004) the antioxidant intake was estimated by dietary history

interview, whereas in the other studies (Mayer-Davis et al. 2002, Song et al. 2005, Nettleton et al. 2006, Wang et al. 2006a) the antioxidant intake was self-reported in a food frequency questionnaire. None of the studies from U.S. showed an association between antioxidant intake (vitamin C, vitamin E, total flavonol, flavones, flavonoids or lycopene) and the risk of incident diabetes. Similarly, in the Finnish Mobile Clinic Health Examination Survey an association was not found between flavonoid intake and risk of incident diabetes for up to 28 years follow-up, as the hazard ratio between the highest and lowest quartile was 0.98 (95% CI 0.77-1.24) (Knekt et al. 2002). Table 4 shows the prospective cohort studies (studies less than 100 cases excluded) of association between intake of antioxidants and risk of type 2 diabetes.

The concentration of antioxidant in plasma or serum may reflect its function more closely, because blood antioxidant concentration is a measure of dietary intake, absorption and also metabolism. In some follow-up studies, an inverse association has been shown between the incidence of type 2 diabetes and serum or plasma concentrations of vitamin E (Salonen et al. 1995, Mayer-Davis et al. 2002) and beta-carotene (Hozawa et al. 2006, Arnlöv et al. 2009, Table 5). In a large study, the European Prospective Investigation of Cancer Norfolk Study, with 21 831 participants and 731 cases of incident diabetes, plasma vitamin C concentration was inversely associated with the risk of incident diabetes during 12 years of follow-up (OR 0.38; 95% CI 0.28-0.52) (Harding et al. 2008). However, other studies have found no association. For example, no protective association was observed between baseline plasma carotenoids and the risk of type 2 diabetes during 10 years of follow-up in the Women's Health Study with 470 cases and controls (Wang et al. 2006b). In the Finnish Mobile Clinic Health Examination Survey no association was found between serum alpha-tocopherol or beta-carotene concentration and the risk of diabetes during 20 years of follow-up (Reunanen et al. 1998). The follow-up studies of baseline serum or plasma antioxidant concentrations and risk of incident diabetes are shown in table 5.

Table 4. Prospective cohort studies of the association between intake of antioxidants and risk of type 2 diabetes.

Reference	Study population/ follow-up time	Cases	Assessment tool	Exposure	Results
Knekt et al. 2002	9878 Finnish women, and men aged 49.2±13.1 years/ 22-28 years	526	Dietary history interview	Total flavonoids, five individual flavonoids	No association
Mayer-Davis et al. 2002	895 U.S. women, and men aged 40-69 years/ 5 years	148	Food frequency questionnaire	Alpha-tocopherol	No association
Montonen et al. 2004	304 Finnish women, and men aged 40-69 years/ 23 years	383	Dietary history interview	Vitamin E, carotenoids, vitamin C	RR[a] 0.69 (95% CI 0.51-0.94) for highest vs. lowest quartile of vitamin E RR 0.58 (95% CI 0.44-0.78) for β-cryptoxanthin, other carotenoids and vitamin C no association
Song et al. 2005	38 018 U.S. women aged ≥45 years/ 8.8 years	1614	Food frequency questionnaire	Total flavonoids, individual flavonols and flavones	No association
Nettleton et al. 2006	35 816 U.S. women aged 55-69 years/ 8 years	3395	Food frequency questionnaire	Total flavonoids, seven flavonoid subclasses	No association
Wang et al. 2006a	35 783 U.S. women aged ≥45 years/ 10.2 years	1544	Food frequency questionnaire	Lycopene	No association

RR, relative risk; CI, confidence interval.

[a]adjusted for age, sex, geographic area, occupation, smoking, body mass index, and family history of diabetes, and energy intake.

Table 5. Follow-up studies of baseline serum or plasma antioxidant concentrations and risk of incident type 2 diabetes.

Reference	Study population/ follow-up time	Cases	Exposure	Results
Salonen et al. 1995	944 Finnish men, aged 42−60 years/ 4 years	45	Lipid standardized plasma alpha-tocopherol	RR = 3.90[b] (95% CI 1.76-8.61) for those with plasma vitamin E below median
Reunanen et al. 1998	106 cases 201 controls[a] Finnish women, men, aged 15−99 years/ 14−20 years	106	Serum alpha-tocopherol and beta-carotene	No association
Mayer-Davis et al. 2002	895 U.S. women, men, aged 40−69 years/ 5 years	148	Plasma alpha-tocopherol adjusted for plasma cholesterol and triglycerides	RR = 0.12[c] (95% CI 0.02-0.68) for highest vs. lowest quintile in supplement non-users
Hozawa et al. 2006	4493 U.S. women, men, aged 18−30 years/ 10−15 years	148	Serum alpha-carotene, beta-carotene, lutein plus zeaxanthin, beta-cryptoxanthin and lycopene	RH = 0.53[d] (95% CI 0.33-0.86)/ SD increase in beta-carotene concentration in non-smokers, no association for others
Wang et al. 2006b	470 cases 470 controls[a], U.S. women, aged 45 years or older/ 10 years	470	Plasma lycopene, alpha-carotene, beta-carotene, beta-cryptoxanthin, lutein / zeaxanthin	No association
Harding et al. 2008	21831 British women, men, aged 40−75 years/ 12 years	735	Plasma vitamin C	OR = 0.38[e] (95% CI 0.28-0.52) for highest vs. lowest quintile
Arnlöv et al. 2009	846 Swedish men aged 50 years/ 27 years	245	Lipid standardized serum alpha-tocopherol, serum beta-carotene	No association for alpha-tocopherol, for beta-carotene OR = 0.68[f] (95% CI 0.53-0.89)/ SD increase in concentration

RR, relative risk; CI, confidence interval, RH, relative hazard; SD, standard deviation; OR, odds ratio.

[a]nested case-control study

[b]adjusted for age, socioeconomic status, body mass index, cigarettes smoked daily, the ratio of serum saturated fatty acids to the sum of monoenes and polyenes.

[c]adjusted for glucose tolerance status at baseline, age, ethnicity, clinic, sex, general health, family history of diabetes, calorie intake, body mass index, waist circumference, smoking status, participation in vigorous physical activity, total fat intake, fiber intake, alcohol intake, and intake of magnesium and vitamin C from food and supplements.

[d]adjusted for race, sex, study center, age, education, systolic blood pressure, ethanol intake, plasma levels of total cholesterol, high density lipoprotein cholesterol, triglycerides, total energy intake, body mass index, total energy expenditure, and use of vitamin supplements.

[e]adjusted for age, sex, family history of diabetes, alcohol consumption, physical activity, smoking, educational level, occupational social class, vitamin supplements, body mass index and waist circumference.

[f]adjusted for body mass index, level of physical activity, smoking status and metabolic model (the baseline covariates for the baseline at age 50 years were impaired fasting glucose, insulin sensitivity [HOMA, homeostasis model assessment], and acute insulin response at intravenous glucose tolerance test [IVGTT]; for the baseline at age 70 years they were impaired fasting glucose, insulin sensitivity [clamp], and early insulin response in the oral glucose tolerance test [OGTT].

2.3.4 Antioxidant supplementation trials on type 2 diabetes and its complications

2.3.4.1 Risk of type 2 diabetes

Only a few randomized controlled trials have studied the effects of antioxidant supplementation on the incidence of type 2 diabetes (Table 6). In the Physicians' Health Study during 12 years of follow-up, 798 cases of incident diabetes were diagnosed in a study population of 21 468 participants. Supplementation with 50 mg of beta-carotene on alternate days had no effect on the risk of incident diabetes (Liu et al. 1999). Likewise, in the Women's Health Study on 1696 cases of incident diabetes in a study population of 38 716 participants, supplementation with 600 IU vitamin E every other day over 10 years of follow-up had no effect on the risk of incident diabetes (Liu et al. 2006). Moreover, no effect of supplemental antioxidants on diabetes risk was found in the Heart Outcomes Prevention Evaluation (HOPE) trial with 261 cases of incident diabetes with a mean follow-up time 4.5 years (Lonn et al. 2002). Two recent studies, the Women's Antioxidant Cardiovascular Study with 895 cases of incident diabetes during a 9.2 year follow-up, and the Selenium and Vitamin E Cancer Prevention Trial (SELECT) with 700 cases of incident diabetes with a median of 5.46 years of follow-up, found no association between supplemental antioxidants and the risk of incident diabetes (Song et al. 2009, Lippman et al. 2009). Similarly, in a French study (the Supplementation en Vitamines et Mineraux Antioxydants, SU.VI.MAX), combination of antioxidants had no effect on fasting plasma glucose in 3146 participants (Czernichow et al. 2006).

Table 6. Placebo-controlled trials of antioxidant supplementation and risk of type 2 diabetes.

Reference	Study population	Supplementation	Follow-up	Cases	Relative risk (95% Confidence interval)
Liu et al. 1999	21 468 men, U.S. aged 40-84 years	Beta-carotene 50 mg on alternate days	Mean 12 years	798	0.99[c](0.86-1.14)
Lonn et al. 2002[a]	5887 women, men 19 countries from Europe, North and South America, aged ≥ 55 years	Vitamin E 400 IU (RRR-alpha-tocopheryl acetate) every day	Mean 4.5 years	261	No effect p = 0.55
Liu et al. 2006	38 716 women, U.S. aged ≥45 years	Alpha-tocopherol 600 IU on alternate days	Median 10 years	1696	0.95[d](0.87-1.05)
Lippman et al. 2009[b]	8737 men, U.S., Canada and Puerto Rico, aged ≥50 years (African-American), ≥55 years (all other men)	Selenium 200 µg (L-selenomethionine) every day, vitamin E 400 IU (all-rac-alpha-tocopheryl acetate) every day, or both	Median 5.46 years	700	Vitamin E 1.04 (0.91-1.18) Selenium 1.07 (0.94-1.22) Vitamin E+ selenium 0.97 (0.76-1.16)
Song et al. 2009	6574 women U.S. aged ≥40 years	Vitamin C 500 mg every day, vitamin E 600 IU (RRR-alpha-tocopherol acetate) every other day, beta-carotene 50 mg every other day	Median 9.2 years	895	Vitamin C 0.89 (0.78-1.02) Vitamin E 1.13 (0.99-1.29) Beta-carotene 0.97 (0.85-1.11)

[a] substudy of the Heart Outcomes Prevention Evaluation (HOPE) trial with 9541 participants
[b] substudy of the Selenium and Vitamin E Cancer Prevention Trial (SELECT)
[c] adjusted for age, aspirin assignment, smoking status, alcohol intake, physical activity, body mass index, history of high cholesterol or hypertension, and use of multivitamins.
[d] adjusted for age and randomized assignment of aspirin and beta-carotene.

2.3.4.2 Complications of type 2 diabetes

Only a few controlled trials about the effects of antioxidant vitamin intakes and the development of complications of type 2 diabetes exist, and their results have not shown discernable effects (Table 7). In the HOPE trial, participants in several countries were randomly allocated to receive either a daily treatment with 400 IU vitamin E or placebo and were followed for a mean of 4.5 years. In all, 3654 people had diabetes at baseline, and vitamin E had no detectable effect on the cardiovascular outcomes (Lonn et al. 2002). In the Medical Research Council/ British Heart Foundation (MRC/BHF) Heart Protection Study on 3982 participants aged 40-80 years with diabetes at baseline, there was no significant effect of antioxidant supplementation on the complications of diabetes (The Heart Protection Study Collaborative Group 2002). In the Women's Health Study, Women's Antioxidant Cardiovascular Study and Physicians Health Study II antioxidants had no effect on the incidence of cardiovascular complications of diabetes (Lee et al. 2005, Cook et al. 2007, Sesso et al. 2008).

Table 7. Placebo-controlled trials of antioxidant supplementation and the risk of complications of type 2 diabetes.

Reference	Country	Study type	Study population	Duration of treatment, years, mean	Daily dose	Results
Lonn et al. 2002[a]	19 countries from Europe, North and South America	Randomized, double-blind	3654 women, men, aged ≥55 years	4.5	Vitamin E 400 IU (RRR-alpha-tocopheryl acetate)	No effect
Heart Protection Study Collaborative Group 2002[b]	United Kingdom	Randomized, double-blind	3982 women, men, aged 40-80 years	5	Beta-carotene 20 mg, alpha-tocopherol 600 mg and vitamin C 250 mg	No effect
Lee et al. 2005[c]	U.S.	Randomized, double-blind	1027 women, aged ≥45 years	10.1	Alpha-tocopherol 600 IU every other day	No effect
Cook et al. 2007[d]	U.S.	Randomized, double-blind, factorial	1564 women, aged ≥40 years	9.4	Vitamin C 500 mg every day, vitamin E 600 IU (RRR-alpha-tocopherol acetate) every other day, beta-carotene 50 mg every other day	No effect
Sesso et al. 2008[e]	U.S.	Randomized, double-blind, factorial	905 men, aged ≥50 years	8	Vitamin C 500 mg every day, alpha-tocopherol 400 IU every other day, multivitamin daily	No effect

Substudy of:
[a]the Heart Outcomes Prevention Evaluation (HOPE) trial with 9541 participants
[b]the Heart Protection Study with 20 536 participants
[c]the Women's Health Study with 39 876 participants
[d]the Women's Antioxidant Cardiovascular Study with 8171 participants
[e]the Physicians' Health Study II with 14 641 participants

3 Aims of the study

Previous studies have shown that obesity and weight gain associate with incident type 2 diabetes, but the findings of a few studies with regard to weight fluctuation and risk of diabetes are inconsistent. Antioxidants have been hypothesized to protect individuals from the development of type 2 diabetes and its complications, but the support for the hypothesis from studies is conflicting.

Accordingly, this study was undertaken to provide more data on the association between weight change, its extent of fluctuation and the risk of diabetes, and to assess the relevance of - both dietary and supplementary antioxidants - on the risk of type 2 diabetes and its complications.

The specific aims of this study were as follows:

1. To study the association between weight change and fluctuation and risk of type 2 diabetes (Study I).

2. To study the association between dietary antioxidants and serum alpha-tocopherol and beta-carotene concentrations and risk of type 2 diabetes (Studies II and III).

3. To study the effect of alpha-tocopherol and beta-carotene supplementation on the incidence of type 2 diabetes (Study III).

4. To study the effect of alpha-tocopherol and beta-carotene supplementation on the incidence of macrovascular complications and mortality in subjects with type 2 diabetes (Study IV).

4 Subjects and methods

4.1 The ATBC Study

The Alpha-Tocopherol, Beta-Carotene Cancer Prevention (ATBC) Study was a randomized, double-blind, placebo-controlled, primary prevention trial (The ATBC Cancer Prevention Study Group 1994). The primary aim was to test the effect of alpha-tocopherol and beta-carotene supplementation on the incidence of lung cancer. The second aim was to evaluate the effects of alpha-tocopherol and beta-carotene on other cancers, all-cause mortality, and incidence of other diseases including atherosclerotic disorders. The study was carried out in 14 areas of southwestern Finland each area with its own study center. The ATBC Study was a joint project between the National Public Health Institute of Finland and the National Cancer Institute, USA. The Institutional Review Boards of the National Public Health Institute, and of the National Cancer Institute approved the ATBC Study. A brief overview of the recruitment, baseline evaluations, interventions and follow-up of the ATBC Study participants is presented. These details have been fully described elsewhere (The ATBC Cancer Prevention Study Group, 1994).

4.1.1 Eligibility, inclusion and exclusion criteria

Participants for the ATBC Study were recruited from the total male population aged 50-69 years living in the study area (n = 290 406) during the 1985-88 period. A postal questionnaire that inquired about smoking habits and willingness to participate in the ATBC Study was sent to all these males. To be eligible, subjects had to smoke five or more cigarettes/day, be willing to participate in the study, and give their written informed consent. In all, 42 957 men were invited to the baseline examination. Exclusion criteria were previous malignancy (other than non-melanoma skin cancer or cancer in situ), severe angina on exertion, chronic renal insufficiency, cirrhosis of the liver, chronic alcoholism, anticoagulant therapy, use of supplements containing vitamin E (over 20 mg/day), beta-carotene (over 6 mg/day) or vitamin A (over 20 000 IU/day), or other medical problems that might limit long-term participation (The ATBC Cancer Prevention Study Group 1994).

4.1.2 Baseline examination and randomization

Men were mailed an invitation letter with a questionnaire on their general background characteristics, medical and smoking histories, level of education, and occupational histories to be filled in at home. Each participant subsequently visited his local study center twice before randomization. During the first baseline visit a nurse checked the questionnaire and completed it when needed. Height and weight of each individual was measured, and his BMI was calculated as the weight in

kilograms divided by square of his height in meters (kg/m^2). Blood pressure was measured by a mercury sphygmomanometer under standardized conditions, and the lower of two measurements taken at least one minute apart was recorded. Overnight fasting blood samples were drawn and the serum stored at -70 ^{o}C. The participants received a referral for chest x-ray and a given food frequency questionnaire to be filled in at home.
Two weeks after the first visit men attended their second baseline visit at which time a study nurse reviewed the food frequency questionnaire. The final study eligibility of each man was then assessed based on the x-ray finding, and the recruited study participants were randomized into the intervention groups in blocks of eight. A total of 29 133 participants were randomized into the trial (The ATBC Cancer Prevention Study Group 1994). All subjects provided their written, informed consent before randomization.

4.1.3 Dietary assessment

Diet was assessed by a food frequency questionnaire (FFQ) specifically designated for the ATBC Study (Pietinen et al. 1988). The questionnaire included 276 food items and mixed dishes with a picture booklet, which contained *inter alia* 122 photographs of foods, each of which had with 3-5 different portion sizes (Pietinen et al. 1988). Each subject was asked to report his usual consumption and the usual portion size of foods during the previous 12 months. A study nurse reviewed and completed the questionnaire together with the subject during the study visit. Then, registered dieticians in the study coordinating centers checked all the questionnaires for final approval. The questionnaire was satisfactorily completed by 27 111 participants (93%). Food consumption data were computed into daily nutrient intake values based on the food composition database at the National Public Health Institute. Vitamin E, vitamin C and carotenoid contents of the foods were based on Finnish analyses (Piironen 1986, Heinonen 1990). In contrast, flavonol and flavone content of foods were based mainly on composition analyses obtained from the Netherlands (Hertog et al. 1992 and 1993) with the exception of the flavonol content of berries, which were based on Finnish analyses (Häkkinen et al. 1999).

Validity of the dietary questionnaire was tested in a pilot study on 189 men by using food records kept for 12 two-day periods as a reference method (Pietinen et al. 1988). The Spearman correlations between the FFQ and food records obtained from these data ranged from: 0.59 to 0.68 for tocopherols, from 0.53 to 0.67 for tocotrienols, from 0.44 to 0.58 for carotenoids, from 0.46 to 0.66 for flavonoids, and 0.55 for vitamin C.

4.1.4 Interventions and compliance

The participants were randomized into groups to receive the following supplemental regimes: alpha-tocopherol (synthetic dl-alpha-tocopherol acetate, 50% powder) 50

mg/day (50 IU/day), or beta-carotene (synthetic beta-carotene, 10% water-soluble beadlets) 20 mg/day, or both or placebo, in a 2 x 2 factorial design. The study capsules were identical gelatine capsules. The active intervention time ended on April 30, 1993 (range 5 to 8 years, median 6.1 years) (The ATBC Cancer Prevention Study Group 1994).

The compliance was calculated by dividing the number of capsules taken by the number of days in the trial, and it was found to be 93% without significant differences between the supplementation groups. About 4% were poor compliers (i.e., took less than 50% of their capsules). Almost all poor compliers dropped out of the study during their first trial year. The overall dropout rate varied only slightly across the four randomized groups (from 30.1% to 30.3%) (The ATBC Cancer Prevention Study Group 1994).

4.1.5 Trial follow-up

The participants visited their local study centers once every four months. Each time the men were asked about their health, medication, use of over-the-counter vitamin supplements, possible side-effects of the trial capsules, and smoking habits (The ATBC Cancer Prevention Study Group 1994). During the study 6 131 (21%) of the participants stopped smoking in approximately equal numbers across the supplementation groups (The Alpha-Tocopherol, Beta Carotene Cancer Prevention Study Group 1994). The used capsule packs were returned during the follow-up visits and a new pack was dispensed. Weight was measured once a year, every twelfth month after commencement of the study up to April 30, 1993. A fasting blood sample was taken from all participants still in the study at the end of the third year (The ATBC Cancer Prevention Study Group 1994).

4.1.6 Laboratory analyses

The baseline and third year serum alpha-tocopherol, beta-carotene and retinol concentrations were determined by high-performance liquid chromatography assay (Milne and Botnen 1986). The between run coefficients of variation (the ratio of the standard deviation to its mean) were 2.2% for alpha-tocopherol, 3.6% for beta-carotene, and 2.4% for retinol. Serum alpha-tocopherol level increased by 50% among the supplement users: baseline median alpha-tocopherol concentration was 11.50 mg/l vs. 17.31 mg/l at 3 years with alpha-tocopherol supplementation. Serum beta-carotene level increased 17-fold: baseline median beta-carotene was 172 µg/l vs. 3001 µg/l at 3 years with beta-carotene supplementation. In the placebo group, serum concentrations were similar at baseline and at three years: median 11.41 mg/l, and 12.40 mg/l, respectively, for serum alpha-tocopherol, and 172 µg/l and 183 µg/l, respectively, for serum beta-carotene (Rapola 1998). Serum total cholesterol and high-density lipoprotein cholesterol (HDL) were determined enzymatically by using the cholesterol oxidase-4-aminophenazone (CHOD-PAP) method (Kostner 1976,

Kattermann et al. 1984). HDL-cholesterol was measured after precipitation of very low-density and low-density lipoproteins with dextran sulphate-magnesium chloride. Glucose in baseline serum samples of identified cases was determined by the enzymatic hexokinase method using an Optima analyzer (ThermoFischer, Vantaa, Finland).

4.2 Subjects

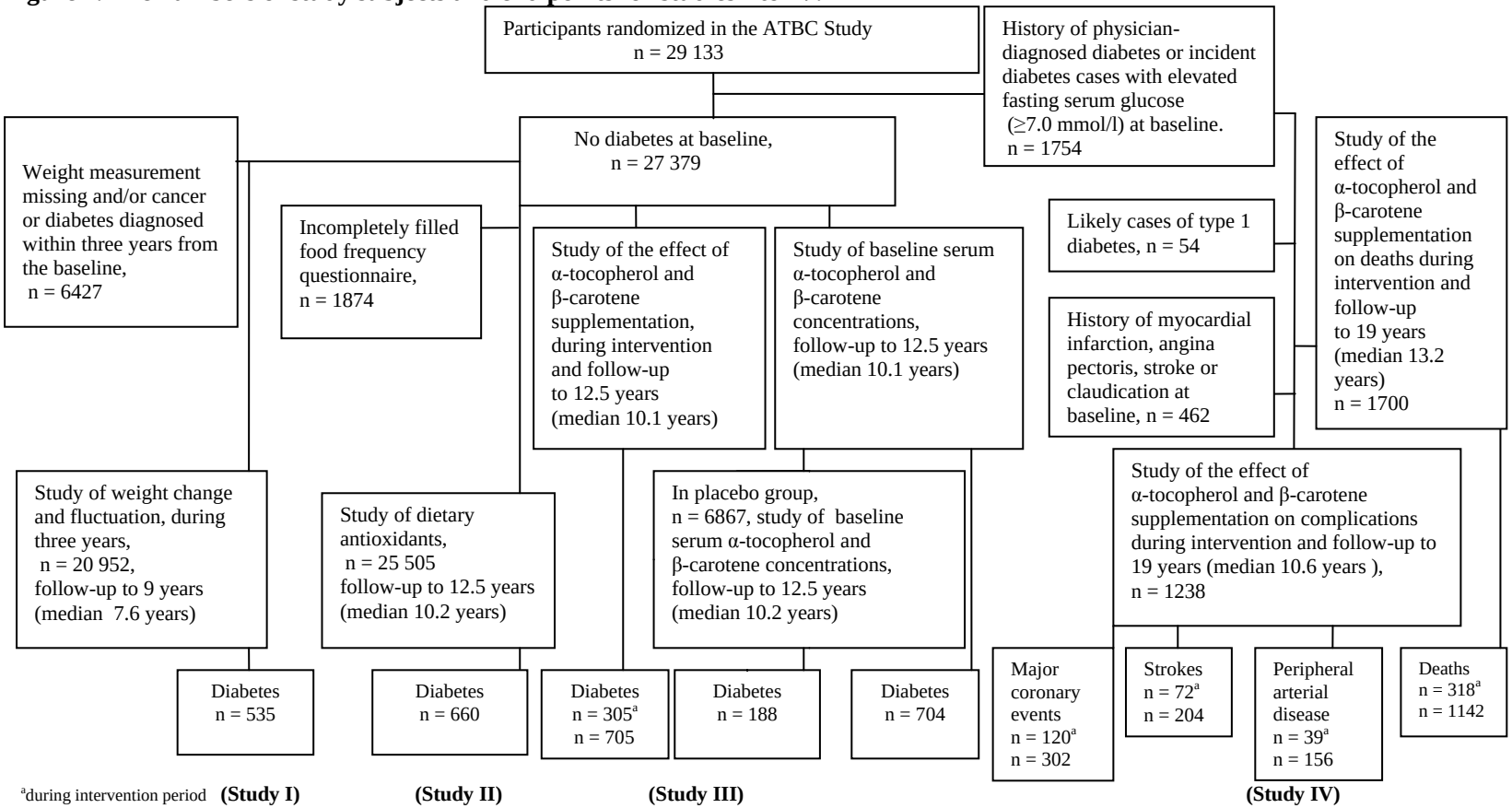
Of the 29 133 men randomized into the ATBC Study, 1754 reported a history of physician-diagnosed diabetes at baseline or had elevated fasting serum glucose (≥7.0 mmol/l) at baseline. For this reason, all these men were excluded leaving a total of 27 379 without diabetes at baseline for the study on the effect of alpha-tocopherol and beta-carotene supplementation on the incidence of type 2 diabetes. Of these, 6867 men belonged to the placebo group, for which the association between baseline serum alpha-tocopherol and beta-carotene and the risk of diabetes was studied. The association between baseline serum alpha-tocopherol and beta-carotene concentrations and the risk of incident type 2 diabetes was also studied in the whole cohort of 27 379 men.

In study on weight change 6427 men were excluded from the study because of one or more of the first four annual weight measurement were missing and/or because they had cancer or diabetes diagnosed within three years of the baseline. Thus, 20 952 men were included in the study of weight change and fluctuation as risk factors for type 2 diabetes.

Of the 27 379 men without diabetes at baseline, 1874 men had incompletely filled in the food frequency questionnaire. Thus, 25 505 participants were included for studying of the relationship between intake of antioxidants and the risk of type 2 diabetes. The men excluded due to incomplete FFQ did not differ significantly in their baseline characteristics from those who had filled in the FFQ satisfactorily except for physical activity during leisure time. Those who had filled in the FFQ satisfactorily participated in moderate or heavy exercise more often than those who filled in the FFQ incompletely (58.8% vs. 54.6%, $p = 0.001$).

Of the 1754 men with a history of diabetes or serum glucose ≥7.0 mmol/l at baseline, 54 were likely cases of type 1 diabetes (insulin as only medication for diabetes) and 462 had a history of physician-diagnosed myocardial infarction, angina pectoris, stroke or claudication. Thus, 1238 participants with diabetes at baseline were included in the study of the effect of alpha-tocopherol and beta-carotene supplementation on macrovascular complications. Figure 1 shows a summary of exclusions, number of subjects and end points in studies I to IV.

Figure 1. The numbers of study subjects and end points for studies I to IV.



4.3 End points

4.3.1 Incident diabetes

All patients in Finland who require a prescription for drug treatment of diabetes are entitled to reimbursement of their medication expenses. This requires a detailed medical certificate issued by the General Practitioner. The Social Insurance Institution checks that the case fulfils the criteria set for diabetes and maintains a register of these cases. At time of the ATBC Study randomization, in the 1980's, a diabetes diagnosis was confirmed when the fasting plasma glucose concentration was 7.8 mmol/l or more, or when the two-hour plasma glucose in an oral glucose tolerance test with 75 g glucose was 11.1 mmol/l or more (WHO 1985). The ATBC Study participants were linked to the register through their respective unique identity numbers, each of which is assigned to every resident in Finland. The incident cases of diabetes through to the end of December 1997 were identified from the register (n = 1187). Cases whose baseline serum glucose was below 7.0 mmol/l (n = 705) were accepted as endpoints for studies I - III, but because of the exclusions shown in Figure 1, the number of incident cases of type 2 diabetes varies in studies I, II and III (Figure 1).

4.3.2 Complications of diabetes

The macrovascular outcomes and deaths were identified through linkage with the National Hospital Discharge Register and the Register of Causes of Death using the codes of the International Classification of Diseases (ICD). These outcomes were identified through to the end of December 2004 and included major coronary event (non-fatal acute myocardial infarction and fatal coronary heart disease, n = 302), total stroke (cerebral infarction, intracerebral haemorrhage and subarachnoid haemorrhage, n = 204) and peripheral arterial disease (atherosclerosis of lower extremities and claudication, n = 156). Major coronary event was sought with ICD-8 and ICD-9 code 410 until 1996 and then ICD-10 codes I21-I23 since 1997 in the Hospital Discharge Register, and with ICD-8 and ICD-9 codes 410-414 and ICD-10 codes I20-I25 from the Register of Death. In a validity study, 94% of the major coronary event diagnoses in the registers were reviewed as a true major coronary event according to strict criteria (Rapola et al. 1997).

Stroke was defined according to ICD-8 or ICD-9 codes 430, 431, 433, 434 and 436 (43101 or 43191, 4330X, 4331X, 4339X excluded) or on ICD-10 codes I60, I61, I63 and I64. About 90% of the stroke cases were cerebral infarction. A validation study of the register diagnoses showed that, according to standard diagnostic criteria, 90% of cerebral infarctions, 79% of subarachnoid hemorrhages and 82% of intracerebral hemorrhages were valid (Leppälä et al. 1999).
Peripheral arterial disease was defined based on ICD-8 codes 44020 and 44390, ICD-9 code 4402A or ICD-10 code I70.2.

4.4 Statistical analyses

The follow-up time extended from the day of the randomization (in the study of the weight change and fluctuation from the date of the fourth annual weight measurement) until the occurrence of the endpoint, death or the end of the follow-up.

The associations between the exposure variables and the incidence of type 2 diabetes and the effect of intervention were both estimated by using Cox proportional hazards regression as relative risks and also 95% confidence intervals. The proportional hazards assumption was tested using the Schoenfeld residuals (Grambsch and Therneau 1994). Stratified analyses were performed in order to assess the possible effect modifications, and the significance of interactions was tested using the likelihood ratio test. Tests for linearity across quintiles were performed using the Wald test by treating the median value of each quintile as a continuous variable. Differences between covariates between subsets was tested using analysis of variance (ANOVA).

In the association analyses the basic model was adjusted for age as continuous variable and supplementation group as categorical variable. The multivariate model was adjusted further for the number of cigarettes smoked daily, years of smoking, systolic and diastolic blood pressure, serum total and HDL cholesterol, BMI and alcohol (ethanol) consumption as continuous variables. In contrast, leisure time physical activity during the past year was treated as categorical (sedentary [e.g. reading, watching television] and moderate [e.g. walking, hunting, gardening] vs. heavy [e.g. running, skiing, swimming]) data in the multivariate models. These leisure time physical activity categories were formed according to participants' own reported estimates. However, these activities were not reported in terms of hours/week.

In the study no. II of the dietary intakes of antioxidants and the risk of diabetes, the multivariate model also included energy intake and in further analyses adjustments were made for the other antioxidants in the same antioxidant group (tocopherols and tocotrienols, carotenoids, flavonols and flavones) as continuous; for the consumption of fruits, vegetables and berries; and coffee consumption as continuous. Protein and saturated fatty acids intake were also regarded as continuous in the multivariate model. The dietary intakes of antioxidants, protein and saturated fatty acids were energy adjusted according to the Willett residual method (Willett and Stampfer 1986, Willett 1990).

In the study no. I of weight change and fluctuation, the average trend in weight change was assessed by analysing the slope of the regression line fitted to four weight measurements taken 1 year apart. The slope was categorized into three classes: a loss of more than 4 kg for weight loss, between - 4 and + 4 kg for stable

weight, and more than 4 kg for weight gain over three years. The 4 kg weight change over 3 years corresponds to an average weight change of 1.33 kg/year.

The association of risk for diabetes with weight fluctuation was examined by calculating the root-mean-square error (RMSE, kg) using annual weight deviations from the corresponding estimated regression line (Lissner et al. 1990, Iribarren et al. 1995, French et al. 1997), and RMSE was divided into quintiles. Weight fluctuation and weight change (loss, stable or gain) were also considered as a combined variable, in which case the weight fluctuation was categorized into two classes: from quintile 1 to quintile 4 (Q1-Q4) for minor fluctuations and quintile 5 (Q5) for large fluctuations.

The intervention analyses were made on the intention to treat dataset. Crude rates per 1000 person years were calculated for the outcomes of each of the four intervention groups (alpha-tocopherol, beta-carotene, alpha-tocopherol plus beta-carotene, placebo) and according to the 2x2 factorial design after testing for no interaction between alpha-tocopherol and beta-carotene. Thus the comparisons were made between participants who received alpha-tocopherol supplements versus those who did not, and participants who received beta-carotene supplements versus those who did not. The intervention-specific cumulative incidence of diabetes was calculated by the Kaplan-Meier method using the log-rank test to calculate the statistical significance of differences between intervention groups. The effect of each supplementation during the follow-up period was expressed by calculating the relative risks at confidence intervals of 95% within consecutive time intervals each of which contained 30 cases of diabetes and then plotting these estimates using 'super smoother' (Friedman 1984).

In the study no. IV on alpha-tocopherol and beta-carotene supplementation and the risk of macrovascular complications of diabetes, the macrovascular outcomes and mortality were analyzed for both the intervention time (i.e. through to April 1993) and the 19-year follow-up period (i.e. through to December 2004). The multivariate model was adjusted for age, number of cigarettes smoked daily, years of smoking, systolic and diastolic blood pressure, serum total and HDL cholesterol, BMI and alcohol consumption as continuous variables and leisure time physical activity as categorical variables.

All analyses were carried out with the R statistical program (R development Core team 2009). All p-values were two-sided, and $p<0.05$ was considered statistically significant.

5 Results

5.1 Baseline characteristics of the main study cohort and their association for the risk of type 2 diabetes

The baseline characteristics were evaluated among the 27 379 participants without diabetes at baseline, (Table 8). The participants who developed diabetes (n = 705) were younger, their serum total cholesterol and HDL cholesterol were lower, daily alcohol use was less than that of the whole cohort, but they had higher BMI and higher systolic and diastolic blood pressures than the whole cohort. The baseline characteristics were equally distributed amongst the supplement groups (Study III).

The multivariate relative risks and 95% confidence intervals for incident type 2 diabetes by baseline characteristics in participants without diabetes at baseline are presented in table 9. Being overweight increased the risk for incident diabetes three fold that of men with normal weight, whereas being obese increased the risk nine-fold ($p<0.001$ for trend). Heavy smoking (26 or more cigarettes a day) was also a risk factor for diabetes. High serum HDL cholesterol was protective for the risk of incident diabetes. Age was not associated with risk of incident diabetes ($p = 0.16$); neither was number of smoking years, nor systolic or diastolic blood pressure, nor daily alcohol consumption or leisure time physical activity.

Table 8. Baseline characteristics of the cohort and cases of type 2 diabetes in the Alpha-Tocopherol, Beta-Carotene Cancer Prevention (ATBC) Study.

Characteristic[a]	Cohort	Cases of diabetes	p-values
Number of subjects	27 379	705	
Age, years	57.1	56.3	= 0.01
Body mass index, kg/m^2	25.8	29.7	<0.01
Number of cigarettes/day	20	20	<0.01
Smoking years	36	36	= 0.14
Systolic blood pressure, mmHg	140	142	<0.01
Diastolic blood pressure, mmHg	88	90	<0.01
Serum cholesterol, mmol/l	6.16	6.12	= 0.27
Serum high density lipoprotein cholesterol, mmol/l	1.16	1.00	<0.01
Alcohol consumption, g/day[b]	11.0	9.3	= 0.02
Leisure time physical activity, %			<0.01
sedentary	41.5	47	
moderate	52.5	49.8	
heavy	6.1	3.3	

[a]medians except leisure time physical activity
[b]n = 25 505 for cohort, n = 660 for cases

Table 9. Multivariate adjusted relative risks for incident type 2 diabetes for baseline characteristics of those ATBC Study participants without diabetes at baseline.

Baseline characteristics			n	Relative risk[a]	95% Confidence interval	p for trend
Age, years		-54	9806	1.00		= 0.16
		55 - 59	8789	1.06	0.87 – 1.28	
		60 - 64	5992	1.24	0.98 – 1.57	
		65 -	2792	1.12	0.79 – 1.59	
Body mass index, kg/m^2	normal	- 24	10 948	1.00		<0.001
	overweight	25 - 29	12 589	3.02	2.31 – 3.93	
	obese	30-	3823	9.29	7.06 – 12.22	
Number of cigarettes/day		-14	5603	1.00		<0.001
		15 - 25	16823	1.17	0.94 - 1.45	
		26-	4953	1.46	1.14 - 1.88	
Smoking years		- 36	13790	1.00		= 0.30
		37-	13533	1.10	0.91 - 1.33	
Systolic blood pressure, mmHg		- 127	6158	1.00		= 0.92
		128 -139	6941	1.03	0.80 - 1.31	
		140- 153	7404	0.95	0.73 - 1.23	
		154-	6873	1.01	0.75 - 1.37	
Diastolic blood pressure, mmHg		- 79	5481	1.00		= 0.15
		80 - 87	7704	0.92	0.71 - 1.20	
		88 – 93	6376	0.96	0.73 - 1.26	
		94-	7814	1.16	0.86 - 1.55	
Serum cholesterol, mmol/l		- 5.43	6710	1.00		= 0.22
		5.44 - 6.14	6826	1.01	0.81 - 1.24	
		6.15 - 6.93	6891	0.97	0.78 - 1.20	
		6.94-	6921	0.88	0.71 - 1.09	
Serum HDL cholesterol, mmol/l		- 0.94	6477	1.00		<0.001
		0.95 -1.11	6832	0.63	0.52 - 0.76	
		1.12 - 1.32	6884	0.52	0.42 - 0.64	
		1.33-	7152	0.47	0.36 - 0.61	
Alcohol consumption[b], g/day		0	2799	1.40	1.11 - 1.76	
		1 -5	15695	1.00		= 0.96
		6 – 36	4946	1.00	0.81 - 1.23	
		37-	2065	0.92	0.67 - 1.25	
Leisure time physical activity, %		light	11347	1.00		= 0.24
		moderate	14357	1.00	0.85 - 1.16	
		heavy	1662	0.64	0.42 - 1.00	

[a]Adjusted for continuous variables age, body mass index, number of cigarettes/day, smoking years, systolic and diastolic blood pressure, total and high density lipoprotein cholesterol, alcohol consumption, and for categorical variables intervention group and leisure-time physical activity.
[b]Alcohol consumption data missing n = 1874

5.2 Weight change and fluctuation and the risk of incident type 2 diabetes

Men who gained at least 4 kg of weight over three years, were slightly younger compared to those men whose weight was stable or who had lost at least 4 kg weight (Table 10). They also had lower serum total cholesterol levels than those whose weight was stable or who had lost at least 4 kg of weight.

Men whose weight fluctuated the most during the subsequent three years (fifth quintile), were younger compared to men in all other quintiles, and they also had lower serum total cholesterol and HDL-cholesterol concentrations than men in other four quintiles (Table 10). They were less physically active during leisure time, and they had higher BMI at baseline than men of the four lowest quintiles.

Weight gain positively associated with the risk of type 2 diabetes, multivariate relative risk was 1.77 (95% CI 1.44-2.17), for weight gain of at least 4 kg per three years compared with those whose weight remained stable (± 4 kg/3 years) (Table 11). Weight loss was not associated with the risk of type 2 diabetes. The relative risks remained similar when the models were adjusted for weight fluctuation. Age modified the association between weight change and the risk for type 2 diabetes ($p = 0.007$ for weight gain and $p = 0.004$ for weight loss) (Table 12). Weight gain was associated with incident type 2 diabetes in participants aged 60 years or older, (RR 3.42; 95% CI 1.70 – 6.88), but not in participants younger than 60 years of age. Moreover, weight loss was associated with incident type 2 diabetes in participants aged 60 years or older (RR 2.07; 95% CI 1.08 – 3.99). The number of cigarettes/day, years of smoking, BMI, alcohol consumption or leisure time physical activity did not modify the association between weight change and the risk for type 2 diabetes.

Weight fluctuation, which was calculated as the root-mean-square error (RMSE, kg) using the annual weight deviations from the corresponding estimated regression line was positively associated with the risk for type 2 diabetes, multivariate adjusted relative risk was 1.64 (95% CI 1.24-2.17) in the highest quintile (>1.67 kg) compared to the lowest quintile (≤0.54 kg) (Table 11). The risk remained similar when adjusted for weight change, relative risk was 1.55 (95% CI 1.17-2.06) for the highest vs. lowest weight fluctuation quintile. Large weight fluctuation increased the risk of diabetes both in men who gained weight (>4 kg), had stable weight (±4 kg), and lost weight (>4 kg) compared to those with stable weight and moderate weight fluctuation (Study I).

Table 10. Baseline characteristics of the participants in categories of weight change and weight fluctuation.

Characteristic[a]	Weight change[b] (kg)			Quintile of weight fluctuation[c] (kg)				
	Loss (>4)	Stable (± 4)	Gain (>4)	Q1 -0.54	Q2 0.55-0.84	Q3 0.85-1.17	Q4 1.18-1.67	Q5 1.68-
Number of subjects	2108	15347	3497	4248	4244	4224	4163	4073
Age, years	57.6	56.9	56.2	57.1	56.9	57.2	56.8	56.4
Body mass index, kg/m^2	27.1	25.6	26.2	25.3	25.5	25.6	26.0	27.1
Number of cigarettes/day	20	20	20	20	20	20	20	20
Smoking years	37	36	35	36	36	36	36	35
Systolic blood pressure, mmHg	142	140	140	140	140	140	140	140
Diastolic blood pressure, mmHg	90	88	88	86	88	86	88	88
Serum cholesterol, mmol/l	6.30	6.20	6.08	6.21	6.20	6.20	6.18	6.16
Serum HDL cholesterol, mmol/l	1.11	1.16	1.16	1.17	1.17	1.16	1.15	1.11
Alcohol consumption, g/day	11	11	11	10	11	11	11	11
Leisure time physical activity, moderate or heavy, %	55	62	57	62	63	62	59	56

Q, quintile
[a]medians or proportions
[b]Weight change is based on the slope of the regression line fitted to the four weight measurements taken one year apart.
[c]Weight fluctuation is the mean deviation of the four weight measurements around the slope (RMSE, root-mean-square error).

Table 11. Number of cases, person years, incidence/1000 person years and relative risk of type 2 diabetes with 95% confidence interval for categories of weight change and weight fluctuation during follow-up of up to 9 years.

Weight variable		Number of cases	Person years	Incidence/1000 person years	Relative risk[a]	95% Confidence interval
Weight change, kg/ 3 years	Loss, >4	71	14 324	5.0	1.17	0.90 – 1.53
	Stable, ±4	310	110 099	2.8	1.00	
	Gain, >4	154	24 688	6.2	1.77	1.44 – 2.17
Weight fluctuation, kg[b]	- 0.54	77	30 344	2.5	1.00	
	0.55 - 0.84	82	30 381	2.7	1.04	0.75 – 1.42
	0.85 - 1.17	90	30 269	3.0	1.13	0.82 – 1.54
	1.18 - 1.67	91	29 679	3.1	1.04	0.76 – 1.42
	1.68 -	195	28 437	6.9	1.64	1.24 – 2.17

[a]Adjusted for continuous variables age, body mass index, number of cigarettes/day, smoking years, systolic and diastolic blood pressure, total and high density lipoprotein cholesterol and alcohol consumption and for categorical variables intervention group and leisure time physical activity.
[b]Weight fluctuation is the mean deviation of the four weight measurements around the slope (RMSE, root-mean-square error).

Table 12. Effect of modification by age on the association between weight change and the risk of type 2 diabetes.

Weight change	Age < 60 years		Age ≥ 60 years		
	Relative risk[a]	95% Confidence interval	Relative risk[a]	95% Confidence interval	p-values for interaction
Loss[b]	0.74	0.55-1.01	2.07	1.08-3.99	p = 0.004
Stable[c]	1.00		1.00		
Gain[d]	1.25	0.90-1.73	3.42	1.70-6.88	p = 0.007

[a]Adjusted for continuous variables age, body mass index, number of cigarettes/day, smoking years, systolic and diastolic blood pressure, total and high density lipoprotein cholesterol and alcohol consumption and for categorical variables intervention group and leisure time physical activity.
[b] >4 kg/3 years
[c] at most ± 4 kg/3 years
[d] >4 kg/3 years

Leisure time physical activities modified the risk for type 2 diabetes with weight fluctuation (p = 0.02). Large weight fluctuations increased the risk for type 2 diabetes in men with light leisure time physical activities (RR 1.95; 95% CI 1.28-2.98) whereas no increase was observed among men with moderate to heavy physical activities during leisure time (Table 13). Furthermore, years of smoking modified the risk for type 2 diabetes (p = 0.04). The risk for diabetes in the highest quintile of weight fluctuation was elevated (RR 1.91; 95% CI 1.27 – 2.88) among men who had smoked less than 36 years. Among participants consuming a mean of 11g or more alcohol daily, weight fluctuation increased the risk more than two times (RR 2.17; 95% CI 1.36-3.45), whereas among those who used less than 11g daily the increase was 58%, (RR 1.58; 95% CI 1.10-2.28), the interaction was significant p = 0.0001 (Table 13). Age, number of cigarettes/day or BMI did not modify the risk for type 2 diabetes with weight fluctuation.

Table 13. Effect of modification by leisure time physical activity, years of smoking and alcohol consumption on the association between weight fluctuation and the risk of type 2 diabetes, relative risks and 95% confidence intervals in five quintiles.

Effect modifier	Weight fluctuation (RMSE quintiles, kg[a])				P for interactions
Quintile	≤0.54	0.55 - 0.84	0.85 - 1.17	1.18 - 1.67	>1.67
Leisure time physical activity[b]:					
Light	1.00	1.18 (0.72 - 1.93)	1.39 (0.87 - 2.24)	1.00 (0.61 - 1.64)	1.95 (1.28 - 2.98) p = 0.02
Moderate or heavy	1.00	0.92 (0.60 - 1.40)	0.93 (0.61 - 1.41)	1.01 (0.68 - 1.51)	1.38 (0.95 - 2.00)
Years of smoking[b]:					
<36	1.00	1.17(0.73 - 1.88)	1.39 (0.88 - 2.19)	1.36 (0.87 - 2.13)	1.91 (1.27 - 2.88) p = 0.04
≥36	1.00	0.94 (0.61 - 1.46)	0.94 (0.61 - 1.45)	0.78 (0.50 - 1.22)	1.43 (0.98 - 2.09)
Alcohol consumption/day[b]:					
< 11 g	1.00	0.80 (0.51 - 1.26)	0.97 (0.63 - 1.50)	1.01 (0.66 - 1.53)	1.58 (1.10 - 2.28) p = 0.0001
≥ 11 g	1.00	1.58 (0.95 - 2.63)	1.39 (0.83 - 2.32)	1.23 (0.74 - 2.07)	2.17 (1.36 - 3.45)

[a] Weight fluctuation is the mean deviation of the four weight measurements around the slope (RMSE, root-mean-square error).
[b] Adjusted for continuous variables age, body mass index, number of cigarettes/day, smoking years, systolic and diastolic blood pressure, total and high density lipoprotein cholesterol and alcohol consumption and for categorical variables intervention group and leisure time physical activity.

5.3 Dietary and serum antioxidants and the risk of type 2 diabetes

Dietary intake of antioxidants was not associated with incident type 2 diabetes (Table 14). Dietary alpha-tocopherol was positively associated with the risk of diabetes when adjusted for age and intervention group though this was not consistent; relative risk in the highest vs. lowest quintile was 1.17 (95% CI 0.91-1.51, p = 0.02 for trend). However, the association disappeared after multivariate adjustment (RR 0.92; 95% CI 0.71-1.19, highest quintile vs. the lowest, p = 0.97 for trend). Similarly beta-tocopherol and beta-tocotrienol were positively associated with the risk of diabetes when age and supplementation were adjusted, but the associations were no longer apparent after multivariate adjusted analyses. Other tocopherols or tocotrienols had no association with the risk of diabetes (Table 14).

Dietary vitamin C or beta-carotene were not associated with the risk of type 2 diabetes. Multivariate adjusted relative risk was 1.04 (95% CI 0.81-1.33) for vitamin C and 1.03 (95% CI 0.79-1.35) for beta-carotene between the highest vs. lowest quintile (Study II). No associations with the risk of diabetes were found with any other dietary carotenoids or any flavonoids (Study II). After further adjusting for other antioxidants in the same antioxidant group, and also for consumption of fruits, for vegetables and berries, for in addition to adjusting for the consumption of coffee, protein and saturated fat the results remained similar. Moreover, the results were not essentially changed when the first five years of follow-up were excluded (Study II). The association between the intakes of antioxidants and the risk of type 2 diabetes was not modified by BMI, leisure time physical activity, or supplementation group.

Baseline serum levels of alpha-tocopherol and beta-carotene were not associated with the risk of type 2 diabetes in the placebo group. Their relative risk in the highest vs. lowest quintile was 1.59 (95% CI 0.89-2.84) for alpha-tocopherol and 0.66 (95% CI 0.40-1.10) for beta-carotene during follow-up to 12.5 years (Study III). Similarly, when studied in the whole cohort (n = 27 379) of the ATBC Study baseline serum level of alpha-tocopherol was not associated with the incidence type 2 diabetes. The relative risk in the fifth quintile compared to the first quintile was 1.08 (95% CI 0.81-1.45) and when quintiles two, three, four and five were combined and compared to quintile one, the relative risk was only 1.04 (95% CI 0.84-1.30) (Table 15).

Baseline serum beta-carotene concentration tended to be associated with lower diabetes risk in the main cohort (n = 27 379). The relative risk in the fifth quintile compared to the first quintile was 0.69 (95% CI 0.52-0.91 (p = 0.01 for trend). However, no significant decreased risk was evident when quintiles two, three, four and five of serum beta-carotene were combined and compared to the first quintile (RR 0.86; 95% CI 0.71-1.04) (Table 15).

Table 14. Relative risk (RR) and 95% confidence intervals (CI) for type 2 diabetes in quintiles of energy-adjusted intake of tocopherols and tocotrienols, during follow-up to 12.5 years.

	Quintile of intake					
	1 Referent	2	3	4	5	p-values for trend
α-Tocopherol, mg/day[a]	6.09	7.34	8.56	10.64	15.47	
Cases	113	113	131	161	142	
RR[b]	1.00	0.97	1.10	1.34	1.17	= 0.02
95% CI		0.74 – 1.26	0.85 – 1.43	1.05 – 1.72	0.91 – 1.51	
RR[c]	1.00	0.97	1.02	1.19	0.92	= 0.97
95% CI		0.74 – 1.26	0.79 – 1.32	0.92 – 1.52	0.71 – 1.19	
β-Tocopherol, mg/day[a]	0.43	0.61	0.78	0.99	1.36	
Cases	115	124	116	150	155	
RR[b]	1.00	1.06	0.98	1.27	1.31	= 0.01
95% CI		0.82 – 1.38	0.76 – 1.28	0.99 – 1.62	1.02 – 1.68	
RR[c]	1.00	1.02	0.89	1.09	1.06	= 0.48
95% CI		0.79 – 1.32	0.68 – 1.17	0.85 – 1.41	0.82 – 1.36	
γ-Tocopherol, mg/day[a]	1.75	3.54	5.91	9.67	18.66	
Cases	117	126	136	133	148	
RR[b]	1.00	1.03	1.08	1.05	1.18	= 0.20
95% CI		0.80 – 1.33	0.84 – 1.40	0.81 – 1.36	0.92 – 1.51	
RR[c]	1.00	0.99	1.07	0.92	0.94	= 0.44
95% CI		0.77 – 1.28	0.83 – 1.37	0.71 – 1.19	0.73 – 1.21	
δ-Tocopherol, mg/day[a]	0.14	0.31	0.54	1.27	4.29	
Cases	119	124	131	135	151	
RR[b]	1.00	0.99	1.00	1.03	1.17	= 0.18
95% CI		0.77 – 1.28	0.78 – 1.29	0.80 – 1.33	0.92 – 1.50	
RR[c]	1.00	0.92	0.96	0.95	0.95	= 0.81
95% CI		0.71 – 1.19	0.74 – 1.23	0.74 – 1.23	0.74 – 1.22	
α-Tocotrienol, mg/day[a]	1.01	1.50	1.89	2.34	3.10	
Cases	137	126	131	125	141	
RR[b]	1.00	0.90	0.94	0.89	1.03	= 0.84
95% CI		0.71 – 1.16	0.73 – 1.20	0.70 – 1.15	0.81 – 1.31	
RR[c]	1.00	0.93	0.91	0.83	1.01	= 0.73
95% CI		0.72 – 1.19	0.71 – 1.17	0.64 – 1.06	0.79 – 1.29	
β-Tocotrienol, mg/day[a]	1.55	2.09	2.50	2.93	3.61	
Cases	116	123	118	151	152	
RR[b]	1.00	1.04	0.99	1.26	1.28	= 0.01
95% CI		0.80 – 1.34	0.76 – 1.28	0.99 – 1.62	1.00 – 1.63	
RR[c]	1.00	0.97	0.89	1.10	1.04	= 0.46
95% CI		0.75 – 1.26	0.68 – 1.17	0.85 – 1.42	0.80 – 1.35	
γ-Tocotrienol, mg/day[a]	0.07	0.12	0.18	0.27	0.45	
Cases	123	125	140	142	130	
RR[b]	1.00	0.99	1.09	1.07	0.98	= 0.89
95% CI		0.77 - 1.27	0.85 - 1.39	0.84 - 1.38	0.76 - 1.26	
RR[c]	1.00	0.99	1.10	1.11	0.90	= 0.73
95% CI		0.77 - 1.28	0.86 - 1.42	0.87 - 1.43	0.70 - 1.17	
δ-Tocotrienol, mg/day[a]	0.01	0.02	0.05	0.09	0.22	
Cases	114	132	125	136	153	
RR[b]	1.00	1.12	1.03	1.07	1.23	= 0.17
95% CI		0.87 - 1.45	0.79 - 1.33	0.83 - 1.38	0.96 - 1.58	
RR[c]	1.00	0.98	1.00	1.02	0.99	= 0.96
95% CI		0.76 - 1.27	0.77 - 1.30	0.79 - 1.32	0.77 - 1.28	

[a]median

[b]Adjusted as continuous for age and intervention group as categorical.

[c]Adjusted for continuous variables age, body mass index, number of cigarettes/day, smoking years, systolic and diastolic blood pressure, total and high density lipoprotein cholesterol, alcohol consumption and energy intake and for categorical variables intervention group and leisure time physical activity.

Table 15. Relative risk (RR) and 95% confidence interval (CI) of type 2 diabetes by quintiles of baseline serum alpha-tocopherol and beta-carotene in the whole cohort of the ATBC Study (n = 27 379) during follow-up of up to 12.5 years.

Serum factor	Quintiles					P- value for trend	Quintiles	P- value for trend
	1	2	3	4	5		2-5	
Alpha-tocopherol, mg/l								
median	8.30	10.10	11.45	13.00	15.71		12.17	
range	0.14-9.31	9.32-10.78	10.79-12.17	12.18-14.06	14.07-127.70		9.32-127.70	
Number of cases	124	139	128	141	172		580	
Person-years	50 089	52 328	53 064	53 270	52 908		211 571	
Adjusted RR[a]	1.00	1.07	0.99	1.05	1.08		1.04	
95% CI		0.83-1.37	0.76-1.29	0.80-1.37	0.81-1.45	p = 0.68	0.84-1.30	p = 0.71
Beta-carotene, µg/l								
median	72	124	173	240	385		203	
range	0-99	100-147	148-202	203-292	293-5686		100-5686	
Number of cases	173	163	154	121	93		531	
Person-years	49 640	51 299	52 635	53 500	54 574		212 008	
Adjusted RR[a]	1.00	0.92	0.92	0.80	0.69		0.86	
95% CI		0.73-1.14	0.74-1.16	0.62-1.02	0.52-0.91	p = 0.01	0.71-1.03	p = 0.10

[a]Adjusted for continuous variables age, body mass index, number of cigarettes/day, smoking years, systolic and diastolic blood pressure, total and high density lipoprotein cholesterol and alcohol consumption and for categorical variables intervention group and leisure-time physical activity.

5.4 Antioxidant supplementation and the risk of type 2 diabetes

The effects of alpha-tocopherol and beta-carotene supplementation on the incidence of type 2 diabetes were studied in cases diagnosed during the intervention period and in cases diagnosed during intervention and 4.5 years post-trial combined (up to December, 1997). Neither alpha-tocopherol alone, nor beta-carotene alone, nor both combined had any effects on the risk of incident type 2 diabetes during the intervention period (Table 16) (Study III). When alpha-tocopherol supplemented were compared to those with no alpha-tocopherol supplementation, and beta-carotene supplemented were compared to those with no beta-carotene supplementation, no effect on the risk of incident diabetes was observed for either supplement and the relative risks were 0.97 (95% CI 0.77-1.22), and 1.01 (95% CI 0.80-1.26), respectively.

The results remained similar when alpha-tocopherol supplemented were compared to non alpha-tocopherol supplemented and beta-carotene supplemented to non beta-carotene supplemented (RR 0.92; 95% CI 0.79-1.07 and RR 0.99; 95% CI 0.85-1.15, respectively) when the follow-up was extended for 4.5 years post-intervention (Table 16) (Study III). After excluding those cases that occurred during the first two years of follow-up, the adjusted relative risk was 0.91 (95% CI 0.78-1.06) for alpha-tocopherol supplemented vs. no alpha-tocopherol supplemented, and 0.99 (95% CI 0.85-1.16) for beta-carotene supplemented vs. no beta-carotene supplemented during the extended follow-up to 12.5 years.

Table 16. Risk of type 2 diabetes in the supplementation groups during intervention period and whole follow-up to 12.5 years.

Follow-up	Treatment	Number of cases/ number of participants at risk	Incidence/1000 person years	Relative risk	95% Confidence interval
Intervention period					
	Alpha-tocopherol	75 / 6862	1.9	0.97	0.70 -1.33
	Beta-carotene	77 / 6832	1.9	1.00	0.72 -1.38
	Alpha-tocopherol + Beta-carotene	75 / 6818	1.9	0.98	0.71 -1.35
	Placebo	78 / 6867	1.9	1.00	
	Alpha-tocopherol vs. no alpha-tocopherol			0.97	0.77-1.22
	Beta-carotene vs. no beta-carotene			1.01	0.80-1.26
Whole follow-up to 12.5 years					
	Alpha-tocopherol	170 / 6862	2.6	0.91	0.73 -1.12
	Beta-carotene	180 / 6832	2.8	0.97	0.79 -1.20
	Alpha-tocopherol + Beta-carotene	167 / 6818	2.6	0.91	0.73 -1.12
	Placebo	188 / 6867	2.8	1.00	
	Alpha-tocopherol vs. no alpha-tocopherol			0.92	0.79-1.07
	Beta-carotene vs. no beta-carotene			0.99	0.85-1.15

5.5 Antioxidant supplementation and the risk of macrovascular complications and mortality in diabetic subjects

Alpha-tocopherol or beta-carotene supplementation, or both in combination, had no effect on the macrovascular complications or mortality in diabetic subjects either during the intervention period, or during the 19-years follow-up of up to December, 2004 (Table 17). When diabetic participants who had received alpha-tocopherol supplement were compared with those who had received no alpha-tocopherol supplement, no effect on the incidence of total macrovascular complications of diabetes was found during the intervention period (RR 0.86; 95% CI 0.65-1.14) or during the extended 19-year follow-up (RR 1.03; 95% CI 0.87-1.21) (Table 18). Similarly, when participants who had received beta-carotene supplement were compared with those who had received no beta-carotene supplement, no effect on the incidence of total macrovascular complications of diabetes was shown either during the intervention period (RR 1.13; 95% CI 0.85-1.51), or during the extended 19-year follow-up (RR 1.05; 95% CI 0.89-1.23).

The incidences of a major coronary event, total stroke and peripheral arterial disease were not significantly different between any of the four intervention groups during either the intervention period ($p = 0.14$, $p = 0.34$ and $p = 0.35$, respectively) or the extended 19-year follow-up ($p = 0.36$, $p = 0.44$ and $p = 0.30$, respectively) (Study IV). The risk of a major coronary event adjusted for major coronary risk factors decreased statistically significantly for those who received alpha-tocopherol compared to those who did not receive alpha-tocopherol (RR 0.64; 95% CI 0.43-0.95) during the intervention period, but during the whole 19-year follow-up the association was non-significant (RR 0.83; 95% CI 0.65-1.06) (Table 18). Beta-carotene supplementation had no effect on the risk of major coronary event.

When alpha-tocopherol supplemented participants were compared to subjects unsupplemented with alpha-tocopherol and beta-carotene supplemented participants were compared to those without beta-carotene supplement, no effects on the risks for total stroke or for peripheral arterial disease were detected (Table 18). However, the risk of stroke was two-fold for those participants with beta-carotene supplementation during the intervention period, who at baseline had diabetes medication (RR 2.02; 95% CI 0.87- 4.69, $p = 0.13$ for interaction). There was no other effect modification factors (BMI, physical activity or hypertension) on macrovascular complications.

There was no significant differences between the four intervention groups during either the intervention period ($p = 0.93$) or the whole 19-year follow-up ($p = 0.32$) for mortality among the diabetic subjects (Study IV). No difference in mortality was

found among those participants who received alpha-tocopherol supplementation compared to subjects unsupplemented with alpha-tocopherol either during the intervention time or up to 19-years follow-up time (RR 1.02; 95% CI 0.81-1.30, and 1.03; 95% CI 0.91-1.17, respectively) (Table 18). Similarly, no difference in mortality was found among those participants who received beta-carotene supplementation compared to subjects with no beta-carotene supplement during either the intervention time (RR 0.98; 95% CI 0.77-1.25), or up to 19-years follow-up (RR 1.03; 95% CI 0.91-1.16). No effect of modification by BMI, physical activity or hypertension was observed.

Table 17. Effects of antioxidant supplementation on the risk of total macrovascular complications and mortality in diabetic subjects during the intervention period and follow-up to 19-years (n = 1238 for incident macrovascular outcomes and n = 1700 for mortality).

Follow-up	Treatment	Number of cases/ number of participants at risk	Incidence/ 1000 person years	Macrovascular complications, total, RR[a] 95% CI, crude	Macrovascular complications, total, RR[a] 95% CI, multivariate adjusted[b]	Number of deaths	Mortality, RR[a] 95% CI, crude	Mortality, RR[a] 95% CI, multivariate adjusted[b]
Intervention period								
	Alpha-tocopherol	51 / 294	31.8	1.00 (0.68-1.49)	1.02 (0.66-1.55)	78	1.04 (0.75-1.45)	1.00 (0.71-1.42)
	Beta-carotene	74 / 331	42.2	1.34 (0.93-1.92)	1.30 (0.88-1.93)	84	1.11 (0.81-1.53)	0.96 (0.68-1.37)
	Alpha-tocopherol+ beta-carotene	54 / 314	31.1	0.98 (0.66-1.44)	0.98 (0.64-1.49)	84	1.06 (0.77-1.47)	1.01 (0.72-1.42)
	Placebo	52 / 299	31.8	1.00	1.00	72	1.00	1.00
19-year follow-up								
	Alpha-tocopherol	159 / 294	52.1	1.12 (0.90-1.41)	1.13 (0.89-1.43)	292	1.17 (0.99-1.39)	1.11 (0.93-1.33)
	Beta-carotene	184 / 331	53.0	1.14 (0.91-1.41)	1.14 (0.90-1.44)	296	1.16 (0.98-1.37)	1.10 (0.92-1.32)
	Alpha-tocopherol+ beta-carotene	161 / 314	48.1	1.03 (0.82-1.29)	1.08 (0.85-1.37)	299	1.13 (0.95-1.34)	1.07 (0.89-1.28)
	Placebo	158 / 299	47.5	1.00	1.00	255	1.00	1.00

[a]RR, relative risk; CI, confidence interval

[b]Adjusted for continuous variables age, body mass index, number of cigarettes/day, smoking years, systolic and diastolic blood pressure, total and high density lipoprotein cholesterol and alcohol consumption and for categorical variable leisure-time physical activity.

Table 18. Effets of alpha-tocopherol and beta-carotene supplementation on the risks for macrovascular outcomes and mortality among diabetic participants during the intervention period and the whole 19-year follow-up (n = 1238 for incident macrovascular outcomes and n = 1700 for mortality).

		Intervention period				19-year follow-up			
		Alpha-Tocopherol	No Alpha-Tocopherol	Beta-Carotene	No Beta-Carotene	Alpha-Tocopherol	No Alpha-Tocopherol	Beta-Carotene	No Beta-Carotene
Macrovascular outcomes, total	Number	105	126	128	103	320	342	345	317
	Rate[a]	31.4	37.2	36.7	31.8	50.0	50.3	50.6	49.7
	RR[b]	0.86	1.00	1.13	1.00	1.03	1.00	1.05	1.00
	95% CI	0.65 - 1.14		0.85 -1.51		0.87 - 1.21		0.89 -1.23	
Major coronary event	Number	49	71	69	51	132	170	151	151
	Rate[a]	14.7	20.9	19.8	15.7	20.6	25.0	22.1	23.7
	RR[b]	0.64	1.00	1.31	1.00	0.83	1.00	0.98	1.00
	95% CI	0.43 - 0.95		0.88 -1.94		0.65 - 1.06		0.77-1. 25	
Total stroke	Number	34	38	43	29	108	96	112	92
	Rate[a]	10.2	11.2	12.3	9.0	16.9	14.1	16.4	14.4
	RR[b]	1.09	1.00	1.21	1.00	1.31	1.00	1.12	1.00
	95% CI	0.64 - 1.86		0.71 -2.08		0.97 - 1.78		0.82 -1.51	
Peripheral arterial disease	Number	22	17	16	23	80	76	82	74
	Rate[a]	6.6	5.0	4.6	7.1	12.5	11.2	12.0	11.6
	RR[b]	1.31	1.00	0.65	1.00	1.16	1.00	1.10	1.00
	95% CI	0.68 - 2.55		0.34 -1.27		0.82 - 1.62		0.78 -1.54	
Total mortality	Number	162	156	168	150	591	551	595	547
	Rate[a]	32.9	32.8	33.9	31.8	56.3	53.2	56.0	53.5
	RR[b]	1.02	1.00	0.98	1.00	1.03	1.00	1.03	1.00
	95% CI	0.81 - 1.30		0.77 -1.25		0.91 - 1.17		0.91 -1.16	

RR, relative risk; CI, confidence interval

[a]Rate per 1000 person-years

[b]Adjusted for continuous variables age, body mass index, number of cigarettes/day, smoking years, systolic and diastolic blood pressure, total and high density lipoprotein cholesterol and alcohol consumption and for categorical variable leisure-time physical activity.

6 Discussion

6.1 Methodological considerations

6.1.1 The ATBC Study population

The ATBC Study was primarily designed to evaluate the effects of alpha-tocopherol and beta-carotene supplementation on the risk of lung cancer and other cancers in a large male population. The trial was based on a 2x2 factorial design, which made the study of the separate effects of each supplement possible. Moreover, the trial enabled the study of the effects of the supplements on other end points such as diabetes data of which were available from national registers.

The ATBC Study protocol included questionnaires on medical histories and lifestyles and measurement of anthropometric characteristics at baseline, which enabled the investigation of the risk factors of diabetes in a cohort setting. These baseline measurements included general risk factors of type 2 diabetes such as BMI, physical activity, blood pressure and serum lipids. An exception to these factors was a family history of diabetes. The annual measurement of weight opened up the possibility to examine the association of a 3-year weight change with the risk for type 2 diabetes.

The participants in the ATBC Study were older middle-aged men with a long history of smoking. Smoking is a risk factor for diabetes (Rimm et al. 1995, Mikhailidis et al. 1998). According to the oxidation hypothesis, it is to be expected that smokers might have benefited most from antioxidant supplementation (Chow 1993). About 21% of the study participants stopped smoking during their active participation in the study (The ATBC Cancer Prevention Study Group 1994). There were, however, no smoking data on individuals after stopping the supplementation and during the post-intervention follow-up. It is possible that those men who stopped smoking were more health-conscious and thus, their dietary antioxidant intakes might have been higher than those of the other participants and being more health-conscious, they might also have kept their weight more stable compared to the other participants. It can also be assumed that they may have undergone more health check-ups and thus it was more likely that they had their diabetes diagnosed than was the case for the less health-conscious men. These factors may have attenuated the association of weight change and antioxidants with diabetes risk towards unity.

Men in the ATBC Study were already at risk of diabetes and atherosclerotic vascular changes at baseline because of their age. In general, background characteristics were

very similar across the supplementation groups. Study subjects represented the male population, but no studies suggest that antioxidants influence males differently than females. The selection process of both the ATBC study program and studies I to IV in this dissertation must be kept in mind when interpreting the results and extrapolating them to other groups of people. Because of several study variables and tests, many significant findings may appear by chance and thus need careful consideration.

6.1.2 Compliance and drop-out

The capsule taking compliance was high with a mean of study subjects taking over 90% of their capsules during their active participation. Only 4% of participants were poor compliers (i.e. took less than 50% of the capsules). The rise in serum alpha-tocopherol and beta-carotene levels measured in the third follow-up year confirmed this good compliance (Rapola 1998). The overall dropout rate during the study was 30.8% for all the intervention groups. Overall drop-out rate varied from 30.1% to 31.3% between the supplementation groups. The commonest causes for drop-out were death and illness 22% and 21%, respectively, followed by no contact (20%) and self-perceived side-effects (14%). The drop-out rate was 11% during the first year, 6% in the second, and then leveled off to about 4% annually (The ATBC Cancer Prevention Study Group 1994).

6.1.3 Assessment of measurements

6.1.3.1 Anthropometric measures

Weight was measured by a trained study nurse, not self-reported by the study participants, during first four annual follow-up visits one year apart to within 100 g accuracy. Each participant wore only light clothing and no shoes during the weighing. Participants had to be compliant for those visits during which the annual weight measurement was planned in order to be included in the study about weight change. Between the measurements there were no data about the weight change and we have no information about whether the weight changes were intentional on the part of the subject. Height was measured at baseline and body mass index for each participant was calculated.

Waist or hip girth was not measured. Waist and hip circumferences measure different aspects of body composition and fat distribution and have independent effects on cardiovascular disease risk factors (Seidell et al. 2001). A narrow waist and wide hips may both protect against cardiovascular disease (Seidell et al. 2001). We categorized weight change as loss, stable or gain. The 4 kg change in weight over 3 years corresponded to a mean weight change of 1.33 kg/year. In the Finnish Diabetes Prevention Study the 2-year net weight reduction, 3.5 kg, was associated with a 58% reduction in diabetes risk (Tuomilehto et al. 2001). The regression slope

fitted to the 4 weight measures over three years was used to assess the trend in weight change. Since there were only four weight measurements taken at intervals of one year, it was not possible to study weight fluctuations that might have occurred over shorter or longer periods of time.

Weight fluctuation was assessed by calculating the root-mean-square error (RMSE, kg) using annual weight deviations from the corresponding estimated regression line. The RMSE estimate may have misclassified those participants who had continuous non-linear weight gain without real weight fluctuation.

6.1.3.2 Dietary and serum antioxidants

The underlying principle of the food-frequency approach was that average long-term diet was conceptually a more important exposure than intakes record on a few specific days (Willett 1990). The result of the food frequency questionnaire (FFQ) is always a very subjective picture of the respondent's own dietary habits and may over-report of healthy food consumption and under-report unhealthy food consumption. In the ATBC Study, the dietary antioxidants were evaluated by using a validated FFQ, which is a standardized epidemiological method (Pietinen et al. 1988). The dietary measurements were recorded at baseline before the diagnosis of diabetes; thus the disease did not influence the recall. At the first visit, the men received the FFQ with instructions and a picture booklet to assess the portion size, which was to be filled in at home. A study nurse used up to half an hour to check the FFQ with each participant. Nutrition professionals revised the questionnaires and made the final acceptance about of the FFQ. In all, 93% of the FFQs were considered satisfactorily completed. The diet was assessed only once at baseline, and thus, dietary changes may have occurred during the follow-up. However, there is no reason to suppose that the dietary changes were dissimilar in the supplementation groups, and thus are unlikely to have had an effect on the risk estimates of the supplementation.

The validity of the dietary assessment instrument as Spearman correlations varied between nutrients from 0.4-0.7, which is acceptable for an epidemiologic study. In validation studies for nutrient intakes, correlation coefficients between 0.5-0.6 are typical (Willett 1990). High correlations between nutrient intakes and that dietary intake reported in an epidemiological study of nutrition may not reflect tissue concentrations accurately may evoke problems (Willett 1990).

A single measurement of blood alpha-tocopherol can represent long-term vitamin E intake to a reasonable degree, and reflect both a capacity to integrate intake over a few weeks and indicate an element of long-term stability of diet among persons (Willett 1990). Nevertheless, plasma lipid content can influence alpha-tocopherol concentration and needs to be taken in account in the analyses (Hunter 1998)

because serum alpha-tocopherol is transported by lipoproteins. However, adjustment for serum cholesterol had no effect on risk estimates of diabetes for serum alpha-tocopherol in this study. A single measurement of blood beta-carotene is also a potentially good index of dietary intake because of its sensitivity to intake and the capacity to integrate over a period of several weeks (Willett 1990). Adjustment for serum cholesterol had no effect on risk estimates for serum beta-carotene.

6.1.4 End points

6.1.4.1 Incident diabetes

The information on incident diabetes was obtained from a nationwide register of drug reimbursements. From the year 1964, patients in Finland with physician diagnosed diabetes are entitled to receive compensation for their diabetes medication from the Social Insurance Institution. According to the Social Insurance Institution records, the drug registry covers over 90% of persons who have medical treatment for type 2 diabetes (Montonen 2005). Therefore, the validity of the diabetes cases obtained from the register of drug reimbursement in Finland is relatively good (Montonen 2005).

The register does not include persons with diabetes who were concurrently undergoing dietary therapy only, which may have led to conservative estimates of the effects and associations between antioxidants and diabetes risk. In 1997, a median of approximately 34% of men with diabetes aged 25-64 years had diet only as a treatment for diabetes as self-reported in a Finnish survey of six urban areas (Korhonen 1999). Each of these areas included 2000 participants (Korhonen 1999). The persons with diabetes treated by diet only and in good metabolic control (HbA1c <6% on controls) often have a milder form of the disease, although they are at increased risk of macrovascular complications (Diabetes 2009). However, so far there are no human studies that show that antioxidants have an association with the risk of type 2 diabetes treated by diet only. There is no reason to assume any causal association between alpha-tocopherol or beta-carotene supplementation and the way of diagnosing incident diabetes cases. Nonetheless, in the cohort study we can assume that for more health-conscious men who undergo more physical examinations, incident diabetes is diagnosed more often than for the less health-conscious men. This difference could have had an effect on the association of antioxidants and the risk of incident diabetes.

The register for drug reimbursement for diabetes contains no data on the type of diabetes. In Finland, approximately 90% of all cases of diabetes are of type 2 (Diabetes 2009). A total, 96% of all diabetic subjects who were diagnosed after the age of 55 had type 2 diabetes in a Finnish study (Laakso and Pyörälä 1985). It is

reasonable to assume that almost all cases would be of type 2 diabetes because all of the new diabetes cases in this study were aged between 50 to 69 years at baseline.

6.1.4.2 Macrovascular end-points and mortality

The National Hospital Discharge Register in Finland includes the dates of hospital admission and discharge, and up to four coded discharge diagnoses, the first of which is the principal cause of the hospital stay. The National Register of Causes of Death contains data on the date and causes of death. A survey of death certificates and cause of death examinations concluded that cause of death statistics of degenerative heart diseases in Finland are reliable (Penttilä and Ahonen 1975). In a Finnish autopsy study, agreement of coronary heart disease antemortem (clinical) diagnoses with autopsy diagnoses was good (Stenbäck 1986).

In a validity study, 94% of the major coronary event diagnoses in the registers were reviewed according to strict criteria as a true major coronary event in the ATBC Study (Rapola et al. 1997). However, there is no reliable register for milder forms of coronary heart disease such as angina pectoris. In the ATBC Study, a validation study of the register diagnoses showed that, according to standard criteria, 90% of cerebral infarctions, 79% of subarachnoid hemorrhages and 82% of intracerebral hemorrhages were valid (Leppälä et al. 1999). By definition claudication is a symptom that is indicative of peripheral atherosclerosis. For this reason, the mildest cases were not retrievable from the above-mentioned registers. However, there was a large number of outcomes identified from national registers, and the long follow-up time enabled any potential long-term effects of antioxidants to be investigated.

Microvascular complications of diabetes are frequent, and are often treated in primary or outpatient care in Finland. However, since there is no reliable register for this type of data it was not possible to study the microvascular complications of diabetes.

6.2 Results

6.2.1 Weight change and fluctuation and risk of type 2 diabetes

In this study a weight gain more than 4 kg over three years, corresponding 1.33 kg weight increase per year nearly doubled the risk of type 2 diabetes during follow-up to nine years. This result of weight gain and increased risk of type 2 diabetes is in line with previous prospective studies which demonstrated gain in weight or body mass index to predict increased risk of type 2 diabetes (Holbrook et al. 1989, Colditz et al. 1995, Hanson et al. 1995, Ford et al. 1997, Resnick et al. 2000, Koh-Banerjee et al. 2004, Oguma et al. 2005, Wannamethee et al. 2005). Even so, direct comparisons are difficult due to differences in how study methods are executed and differences between study populations. Among middle-aged and elderly male health

professionals weight gains of at least 9 kg over 10 years resulted in a relative risk of 2.10 compared to those with weight increase or loss not exceeding 2 kg during a subsequent follow-up of four years. Nonetheless, it should be noted that in that same study a 3-5 kg increase of weight was associated with an increased risk of type 2 diabetes, relative risk 1.40 (Koh-Banerjee et al. 2004). An even higher risk (RR 5.50; 95% CI 4.70-6.30) during follow-up of 14 years was found in participants of the Nurses' Health Study who gained at least 11 kg of weight since aged 18 years (Colditz et al 1995). Surprisingly, in the NHEFS Study a weight gain of 8-10 kg in about 10 years was associated with a non-significant relative risk (RR 1.19; 95% CI 0.75-1.89) during subsequent follow-up of 8-10 years compared with those whose weight had changed less than 5 kg (Ford et al. 1997). When the weight gain was as much as 11 to 19 kg in the same study, the relative risk was 2.66 (95% CI 1.84-3.85) and even higher among those who gained weight at least 20 kg.

Age *per se* was not a risk factor for type 2 diabetes in this study, but age was a modifying factor in the association of weight gain and the risk of diabetes. Among those who were older than 60 years of age at baseline, weight gain was significantly associated with a higher risk for diabetes than among those of stable weight. Among those who were younger than 60 years at baseline, no such an association was observed. Obesity and older age are causes of insulin resistance and may partly explain this association (Moller and Flier 1991). Moreover, metabolically active increased abdominal fat (Shulman 2000) in relation to diminishing muscle mass may also be a contributing factor.

Importance of weight loss in decreasing type 2 diabetes risk is clearly demonstrated in intervention studies aimed at decreasing disease risk (Tuomilehto et al. 2001, Davey Smith et al. 2005, Hamman et al. 2006). A weight loss of at least 4 kg per three years which equates to a mean annual loss of 1.33 kg also corresponds to a 2-year weight reduction of 2.66 kg which approximates to the reduction that was associated with a 58% reduction in diabetes risk in the Finnish Diabetes Prevention Study (Tuomilehto et al. 2001). However, in this prospective study weight loss did not predict decreased diabetes risk. This result also differs from the findings of other prospective cohort studies, which have associated weight losses with decreased risks of type 2 diabetes (Colditz et al. 1995, Resnick et al. 2000, Will et al. 2002, Koh-Banerjee et al. 2004, Wannamethee et al. 2005). These studies include a finding from the U.S. men and women who were overweight at baseline (Resnick et al. 2000) and also U.S. men and women who reported intentional weight loss at baseline (Will et al. 2002). In two large population based studies one on the Nurses' Health Study cohort (Colditz et al. 1995) and the other on middle-aged and elderly male health professionals (Koh-Banerjee et al. 2004), the participants because of their profession were apparently more aware of healthy lifestyle, which might partly explain their results.

That the results of the present study for weight loss and the risk of incident type 2 diabetes differed from above-mentioned studies may also have other explanation. Participants in the ATBC Study were middle-aged to older male smokers with an increased risk for atherosclerosis and cardiovascular diseases. Although men with previous malignancy, severe angina on exertion, chronic renal insufficiency, cirrhosis of the liver, chronic alcoholism, and anticoagulant therapy were excluded prior to randomization, several other acute or chronic conditions could have occurred during the intervention and follow-up periods which cause abnormal physiologic states. Therefore, weight loss may have been due to emerging or chronic ill health. It is possible that some underlying devastating disease may have affected the emerging diabetes. Moreover, there are several previous studies that also reported no significant decrease in type 2 diabetes risk upon weight loss (Ford et al. 1997, Ishikawa-Takata et al. 2002, Oguma et al. 2005, Mishra et al. 2007, Jacobs-van der Bruggen et al. 2010, Waring et al. 2010). In one prospective study weight loss was even associated with increased risk of type 2 diabetes (Holbrook et al. 1989).

Although weight loss was not associated with an increased risk in the whole study population, age was found to be a modifying factor for the association. Weight loss predicted increased diabetes risk among older men (≥60 years), but was associated with marginally decreased risk among men under 60 years of age. Insulin stimulates the uptake, storage and use of glucose in muscle (Moller and Flier 1991). Aging is associated with significant changes in body composition, including the loss of skeletal muscle mass and increased visceral fat accumulation (Johannsen and Ravussin 2010). Aging is also characterized by a drop in resting metabolic rate that is disproportionate to the loss of muscle mass, with an associated shift towards preferentially oxidizing carbohydrate at the expense of fat. A combination of these factors may act to increase muscular lipid infiltration and decrease insulin sensitivity (Johannsen and Ravussin 2010).

Weight fluctuation estimated over three years was associated with an increased risk for type 2 diabetes; the relative risk in the highest quintile was 1.6 fold compared with the lowest quintile of weight fluctuation measurement. This result is in line with two previous prospective follow-up studies (French et al. 1997, Brancati et al. 1999), whereas in three other studies no independent association was found between weight fluctuation and the risk of type 2 diabetes (Hanson et al. 1995, Field et al. 2004, Waring et al. 2010). In the Johns Hopkins Study the participants were U.S. men aged 50 years. Their BMI variability from ages 20 to 49 years more than doubled their risk for type 2 diabetes (RR 2.1; 95% CI 1.0-4.6) during the mean follow-up of 15.6 years (Brancati et al. 1999). In that study sample, the number of participants was 916 with only 35 cases of incident diabetes. In another study among middle-aged and elderly U.S. women, large weight fluctuations were estimated over decades of adult life, and the relative risk for incident type 2 diabetes was found to

be 1.70 (95% CI 1.25-2.29) during a follow-up of six years (French et al. 1997). In a study on adult women severe weight cycling associated with intentional weight loss over four years predicted an elevated risk of type 2 diabetes, but this association was not independent of body mass index (Field et al. 2004). In a recent study of middle aged men and women weight cycling during middle age was not associated with higher rates of type 2 diabetes after adjustment for weight status and weight change (Waring et al. 2010). In the present study weight fluctuation estimated over a three year period and based on measured body weight was associated with an increased risk of diabetes independent of body mass index and of weight change. This finding is new, but needs to be confirmed with other studies with different populations, more measurements of body weight, and different follow-up times. The root-mean-square error (RMSE) measurement used to estimate weight fluctuation during the three year period has its restrictions. The main restriction is that there were no data on the variation of weight for a period more than three years.

Leisure time physical activity modified the association between weight fluctuation and risk of type 2 diabetes. When only light exercise was taken, large weight fluctuations increased the risk of diabetes two-fold, whereas no association was found when the exercise was moderate or heavy. Regular exercise has been found to cause several adaptations in the body improving the muscle and whole body insulin sensitivity (Perseghin et al. 1996).

6.2.2 Antioxidants

6.2.2.1 Dietary and serum antioxidants and the risk of type 2 diabetes

In this study dietary intake of antioxidants did not have an association with risk of incident type 2 diabetes. Thus, these findings do not support the hypothesis that dietary antioxidants prevent diabetes. The results are in line with earlier prospective cohort studies for which antioxidant intake was found to have no association with the risk of incident type 2 diabetes (Knekt et al. 2002, Mayer-Davis et al. 2002, Song et al. 2005, Nettleton et al. 2006, Wang et al. 2006a). To date, only one study has shown that antioxidant intake is associated with a reduced risk of type 2 diabetes (Montonen et al. 2004). In their study the risk of type 2 diabetes was 30% lower among persons in the highest vs. lowest quartile of vitamin E intake. Furthermore, among the carotenoids considered in their study, beta-cryptoxanthin intake was inversely associated with diabetes risk (Montonen et al. 2004). One limitation in that study is that no valid data on baseline physical activity were available. It may also be possible, that individual antioxidants are only markers for a more healthy diet. In the Finnish Mobile Clinic Health Examination Survey population the dietary pattern which was characterized by higher consumption of fruits and vegetables was inversely associated to type 2 diabetes risk (Montonen et al. 2005).

The circulating levels of antioxidants in the body may better reflect the capacity of the body to combat against oxidants. However, baseline serum levels of alpha-

tocopherol and beta-carotene were not associated with the risk of incident type 2 diabetes in the placebo group or in the whole cohort in this study. These results are in line with those of three other studies; the Finnish Mobile Clinic Health Examination Survey (Reunanen et al. 1998), the Uppsala Longitudinal Study of Adult Men for serum alpha-tocopherol (Arnlöv et al. 2009), and the Women's Health Study for plasma carotenoids (Wang et al. 2006b). The findings of the prospective studies are, however, contradictory. In two prospective observational studies serum alpha-tocopherol levels were associated with lower risks of diabetes (Salonen et al. 1995, Mayer-Davis et al. 2002). However, in the Kuopio Ischemic Heart Disease Risk Factors Study which studied 944 men, there were only 45 cases of incident diabetes (Salonen et al. 1995). Serum beta-carotene concentration was associated with reduced risk of incident diabetes in two studies (Hozawa et al. 2006, Arnlöv et al. 2009). In these studies the impacts of serum lipids on alpha-tocopherol and beta-carotene values were taken into account by utilizing lipid standardized serum values or by using an adjustment for serum cholesterol in the analyses. Accordingly, differences in the consideration of serum lipids do not seem to explain dissimilar results.

6.2.2.2 Intervention effects

6.2.2.2.1 Risk of type 2 diabetes

Supplementation of alpha-tocopherol or beta-carotene separately or in combination had no effect on the risk of incident type 2 diabetes during either the intervention period with a median follow-up time of 6.1 years, or during the whole follow-up which extended for 4.5 years after the intervention. The results of the present study support the few previous controlled clinical trials which found no effect with antioxidant supplementation on the risk of type 2 diabetes. The dosages of the supplements were as follows: in the Physician' Health Study the supplementation dose was 50 of mg beta-carotene on alternate days (Liu et al. 1999), in the HOPE Study 400 IU vitamin E a day (Lonn et al. 2002), in the Women's Health Study 600 IU vitamin E on alternate days (Liu et al. 2006), in the Women's Antioxidant Cardiovascular Study 500 mg synthetic vitamin C every day, 600 IU vitamin E or 50 mg beta-carotene every other day (Song et al. 2009), and in the SELECT Study 200 µg selenium every day or 400 IU vitamin E every day, or both (Lippman et al. 2009). In previous studies relative risk for those receiving vitamin E supplement compared to placebo group varied between 0.95 (95% CI 0.87-1.05) (Liu et al 2006) and 1.13 (95% CI 0.99-1.29) (Song et al. 2009). Relative risk for beta-carotene supplement receivers compared to those without supplement was 0.97 (95% CI 0.85-1.11) in one study which investigated the effect of beta-carotene supplementation (Song et al. 2009). Relative risk in another study was 0.99 (95% CI 0.86-1.14) with the same dosage of beta-carotene supplement compared to non-receivers of beta-carotene supplement (Liu et al. 1999).

Although the results of the present study are in line with the above-mentioned studies there are also dissimilarities between these studies. In the ATBC Study the participants were male smokers, whereas in the Physicians' Health Study the participants were possibly more health-conscious men who had healthy diet habits and life styles. At baseline, 7.1% of the men in Physicians' Health Study smoked ≥20 cigarettes per day (Liu et al. 1999). In the Women's Health Study 13% of the participants of the vitamin E intervention group were current smokers at baseline (Liu et al. 2006). In most of the studies daily supplements of antioxidants, especially vitamin E, were considerably higher than in the ATBC Study and some studies used different combinations of antioxidants (Lippman et al. 2006, Song et al. 2009). The results of these studies support the conclusion that vitamin E or beta-carotene as supplements are not preventive for type 2 diabetes.

6.2.2.2.2 Complications of type 2 diabetes

This study observed that macrovascular complications of diabetes or mortality from diabetes were not influenced by antioxidant supplementation. These results are in line with the previous randomized double-blinded and placebo-controlled studies which had up to 10.1 years mean follow-up time for which neither vitamin E, vitamin C, and beta-carotene had no detectable effects on reducing the macrovascular complications of diabetes or total or cardiovascular mortality (Lonn et al. 2002, The Heart Protection Study Collaborative Group 2002, Lee et al. 2005, Cook et al. 2007, Sesso et al. 2008). The dosage of vitamin E supplementation (alpha-tocopherol 50 mg/day [50 IU]) was low in the ATBC Study compared to above-mentioned studies. In the HOPE Study and in the Physicians' Health Study II the daily dose of vitamin E was 400 IU daily or every other day (Lonn et al. 2002, Sesso et al. 2008), and in the Women's Antioxidant Cardiovascular Study and the Women's Health Study the vitamin E dose was 600 IU daily or every other day, respectively (Lee et al. 2005, Cook et al. 2007).

The daily dose of beta-carotene (20 mg/day) in the ATBC Study is in line of the supplementation dosage of the MRC/BHF Heart Protection Study (The Heart Protection Study Collaborative Group 2002). In the Women's Antioxidant Cardiovascular Study the dosage of beta-carotene was 50 mg, but taken every other day (Cook et al. 2007). The populations of these intervention studies were drawn from different countries in Europe, North America and South America and they included both female (Lonn et al. 2002, The Heart Protection Study Collaborative Group 2002, Lee et al. 2005, Cook et al. 2007) and male participants (Lonn et al. 2002, The Heart Protection Study Collaborative Group 2002, Sesso et al. 2008). All these earlier studies and this study with different populations and varying dosages of supplemented antioxidants conclude that the supplementations with antioxidants do not decrease the risk of macrovascular complications or mortality of persons who have type 2 diabetes.

7 Conclusions

The ATBC Study demonstrated that weight change and fluctuation were independent risk factors for type 2 diabetes, in middle-aged and older smoking Finnish men. Furthermore, this study demonstrated that type 2 diabetes was not preventable by dietary antioxidants or by antioxidant supplementation (alpha-tocopherol or beta-carotene). Antioxidant supplementation (alpha-tocopherol or beta-carotene) did not prevent macrovascular complications of diabetes nor total mortality of diabetics.

The conclusions responding to the objectives of this study are:

1. Weight gain was a risk factor for type 2 diabetes in middle-aged and older smoking men. This result is in line with the results in previous prospective cohort studies. Weight gain was a risk factor for type 2 diabetes especially in men aged 60 years or older, as was weight loss for persons aged 60 years or older. Weight fluctuation during a three year period was associated with increased risk for type 2 diabetes independent of weight gain. This finding is new and needs to be confirmed in future studies.

2. Intake of dietary antioxidants - tocopherols, tocotrienols, carotenoids, vitamin C or flavonoids - at baseline was not associated with the risk of type 2 diabetes. Similarly, high baseline serum alpha-tocopherol or beta-carotene levels were not associated with lower risk of incident type 2 diabetes in the placebo group or in the whole study cohort .

3. Neither alpha-tocopherol nor beta-carotene supplementation prevented type 2 diabetes when analyzed either during the intervention period or during the whole 12.5 year follow-up of this study.

4. Neither alpha-tocopherol nor beta-carotene supplementation decreased the incidence of macrovascular complications of diabetes or total mortality of diabetic men either during the intervention period or during the whole 19-year follow-up in this study.

The primary aim of this study was to evaluate whether serum or dietary antioxidants or supplemented antioxidants (alpha-tocopherol or beta-carotene) have any effect on preventing type 2 diabetes or macrovascular complications of diabetes or mortality

of diabetic subjects. In line with the negative results from previous controlled trials these results give no reason to suggest supplementation with beta-carotene or alpha-tocopherol for the prevention or treatment of diabetes. Avoiding weight gain and weight fluctuation decreased the risk of type 2 diabetes. Consequently, a healthy lifestyle with a diverse diet, which keeps the body weight stable are to be recommended to the public as measures to prevent type 2 diabetes.

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